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Wanneer ben jij op je best?

Gezondheid is een groot goed. Hier is iedereen het over eens. Iedere dag zetten veel mensen zich in voor de Nederlandse gezondheidszorg. Ieder op zijn/haar manier. Artsen, verplegers, apothekers, medewerkers van geneesmiddelenbedrijven en alle andere zorgverleners. Je kan zelf ook werken aan je gezondheid door onder andere voldoende te bewegen, niet te roken, weinig alcohol te nuttigen, gezond te eten en voldoende nachtrust te nemen. Probeer ook niet te veel te stressen over stress.

Onze expertise is het ontwikkelen van betekenisvolle en vernieuwende geneesmiddelen, waaronder biosimilars (patentloze biologische geneesmiddelen) en vaccins.

We streven hierbij naar grensverleggende innovaties. Dit is meer dan ons werk. Het is onze passie. Of het nu gaat om individuele zorg, veel voorkomende of juist erg zeldzame ziektes.

Maar hoe jij je voelt... dat is minstens net zo belangrijk. Want uiteindelijk wil iedereen niet alleen zo gezond mogelijk zijn, maar zich ook graag zo goed mogelijk voelen. Op zijn/haar best. Om zo een gelukkig, volwaardig en rijk leven te leiden.

Wij zijn op ons best als we ons iedere dag weer kunnen richten op doorbraken die de levens van patiënten veranderen. Wanneer ben jij op je best?



Doorbraken die de levens van patiënten veranderen

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Exosomal release of the human cytomegalovirus-encoded chemokine receptor US28

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Introduction

Exosomes are nanosized extracellular vesicles (EVs) that originate from the fusion of multivesicular bodies (MVBs) with the plasma membrane. These vesicles mediate intercellular communication via the transfer of proteins and RNA. In the last decade, it has become clear that many viruses, including herpesviruses, modulate exosomal communication to create an optimal environment for viral persistence and dissemination. In this study, we show that the human cytomegalovirus (HCMV)-encoded chemokine receptor US28 is released via exosomes. This viral receptor is expressed in both latent and lytic stages of HCMV infection and constitutively activates proliferative and pro-angiogenic signaling pathways. We hypothesize that exosomal release of US28 might contribute to HCMV pathology.

Methods

We developed an optical reporter based on US28 and a pH-sensitive GFP (pHluorin) that enables live cell imaging of the fusion of US28-containing MVBs with the plasma membrane. Furthermore, we generated a HCMV strain containing US28-pHluorin to study exosomal release of US28 in HCMV-infected cells.

Results

Live cell TIRF microscopy on HCMV-infected cells revealed that US28-pHluorin-containing MVBs fuse with the plasma membrane. In line with this, EVs isolated from the culture supernatant of infected cells contain US28. Moreover, analysis of the EV-fraction by super-resolution STED and electron microscopy confirmed the presence of US28-pHluorin-positive EVs with a diameter of 50-100 nm, corresponding to the size of exosomes.

Summary/conclusion

Together, these results suggest that HCMV-infected cells release US28 via exosomes. In future studies, the US28-pHluorin system can be used to study the functional consequences of US28 exosome release and to identify potential strategies to block exosomal communication by HCMV.

RePAIR: a power solution to animal experimentation

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Recent evidence from animal studies, including our own extensive analysis based on >1500 studies, shows that the median power is around 0.2, rather than the often assumed power of 0.8.

This poses a serious problem, which may have substantially contributed to the fact that new treatment options developed in animal models frequently failed in clinical trials in humans. One solution would be to increase the number of animals per study, i.e. from the current (median) 10 to 20-400 per group, depending on the research question. In view of the current views on the use of laboratory animals, this may not be the way to go. Therefore, we developed *RePAIR* (*Reduction by Prior Animal Informed Research*), a hybrid Bayesian-frequentist statistical method, that uses previously obtained information to increase the power of new studies. By using information from previous experiments (prior), we first show in a simulation model that *RePAIR* can increase the power of the test performed by up to ~100%; or, alternatively, help to decrease the N of the control group by ~49% of the total N. The underlying assumption is that all control animals belong to the same population. In data collected on a particular behavioral test in >250 rodents we show that this assumption is indeed warranted. *RePAIR* comes with an open-source web-based tool to ease its implementation.

Quantifying the morphine concentration effect relationship for postoperative pain in children using item response theory modelling

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Introduction

Despite the routine use of morphine to treat postoperative pain after major surgery in children, a quantitative understanding of its concentration-effect relationship is still lacking for this population. In preverbal children, pain is assessed with instruments like the COMFORT-behavior (COMFORT-B) that quantify pain-associated behavior. Using item response theory models to analyze data from composite scales like the COMFORT-B can increase statistical power compared to analyzing total score data.

Objectives

The study objective was to quantify the concentration-effect relationship of morphine for the treatment of postoperative pain in children using the COMFORT-B item-level data.

Methods

A total of 2402 COMFORT-B assessments after major non-cardiac surgery in 207 children between 0 and 3 years old was available from two prior clinical studies.^{1,2} Children received an intravenous morphine loading dose of 100 mcg/kg at the end of surgery, followed by intravenous morphine or acetaminophen in 200 (97%) and 7 (3%) of the children, respectively. Rescue morphine administrations were guided by a standardized pain protocol.

From the individual item-level data of COMFORT-B assessments, an item response theory model was developed to estimate a latent variable representing the level of pain. A pharmacokinetic-



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pharmacodynamic model was developed in NONMEM 7.3 to characterize the effect of morphine and patient characteristics on this latent variable.

Results

With respect to the morphine concentration effect relationship, a population of responders (73%) and non-responders (27%) was found ($p < 0.001$). For the responders, the concentration-effect curve of morphine is relatively flat between 5 and 15 ng/ml, with increasingly lower COMFORT-B scores at concentrations above 20 ng/ml (Figure 1).

Conclusion

Here, we used item response theory modelling quantify the concentration-effect relationship of morphine for postoperative pain in children. The results suggest that in children that are uncomfortable (COMFORT-B above 17) at morphine concentration between 5 and 15 ng/ml, dose escalation to morphine concentrations above 20 ng/ml might be required to reach the steeper part of the concentration-effect curve. Children that do not respond to morphine, might be distressed or undersedated, rather than in pain.

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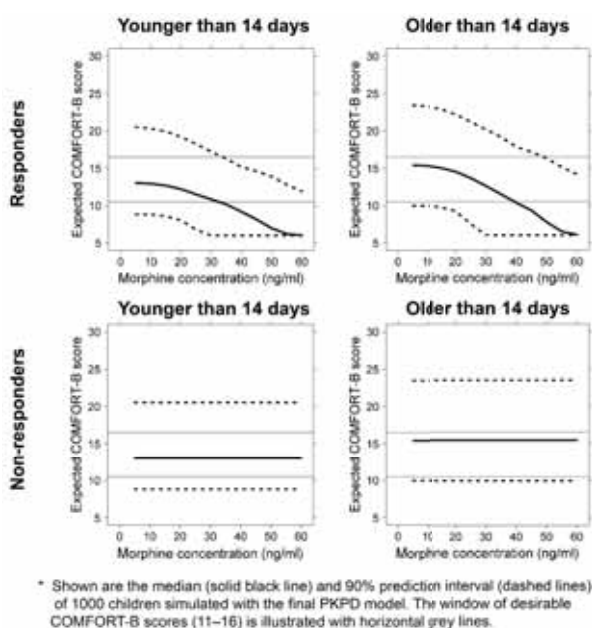


Figure 1. Concentration-effect relationship of morphine in responding children after major non-cardiac surgery.

Exposure and response analysis of aleglitazar on cardiovascular risk markers and safety outcomes: An analysis of the AleCardio trial

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Background

The AleCardio trial aimed to characterize the efficacy and safety of PPAR α agonist aleglitazar in patients with type 2 diabetes mellitus and acute coronary syndrome. The trial terminated early due to futility and safety signals. We evaluated whether the safety signals could be attributed to increased exposure to aleglitazar.

Methods

The AleCardio trial enrolled 7226 patients to receive aleglitazar 150 μ g or matching placebo on top of standard of care. Plasma samples were collected in a pharmacokinetic sub-study in 515 patients of the aleglitazar arm. A population pharmacokinetic analysis was conducted on the sub-study to identify covariates that explained interindividual variability in exposure. Subsequently, the effect of these covariates on surrogate and clinical outcome was assessed in the full patient population.

Results

Concomitant administration of clopidogrel was identified as a covariate that influenced the apparent clearance of aleglitazar. Patients using clopidogrel had a mean predicted AUC₀₋₂₄ of 142.2 ng·h/ml (SD: ± 92.6 ng·h/ml) versus 174.7 ng·h/ml (SD: ± 112.9 ng·h/ml) in patients without clopidogrel. The effect of aleglitazar compared to placebo on HbA_{1c}, hemoglobin, serum creatinine and adiponectin was modified by concomitant clopidogrel use (p for interaction 0.007, 0.002, <0.001 and <0.001 respectively).

Discussion

Concomitant use of clopidogrel was identified as covariate that explained interindividual variability in exposure to aleglitazar. Patients using clopidogrel demonstrated an additional lowering of HbA_{1c}, at the expense of an additional decrease in hemoglobin and increase in serum creatinine and adiponectin. Clopidogrel is a moderate inhibitor of CYP2C8. Since aleglitazar is metabolized by CYP2C8, a pharmacokinetic interaction could explain interindividual differences in exposure and response to aleglitazar in the AleCardio trial.

The influence of external factors on the pharmacokinetics of oral targeted anticancer drugs Summary

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The intake of oral targeted anticancer therapies with food and concomitant use of gastric acid suppressing agents (GAS) can significantly affect drug absorption.¹

Aims

Patients using pazopanib, an oral targeted anticancer drug, often experience gastro-intestinal (GI) toxicities.^{2,3} We hypothesized that ingesting pazopanib with food may improve patients'

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comfort and reduce GI-toxicity. In addition, it was demonstrated that the use of pazopanib with GAS resulted in shorter overall survival and progression free survival in patients with soft tissue sarcoma.⁴ In order to limit the effect of GAS on pazopanib absorption, the advice is to take the GAS 1 hour after pazopanib while the effect of this advice is unknown. Therefore, we aimed to investigate the effect of food and the effect of the controlled intake with GAS (i.e. the GAS was taken 1 hour after pazopanib) on pazopanib exposure.

Methods

In a multi-center open label randomized trial (DIET study) we investigated the bioequivalent dose of pazopanib when taken with food compared to the standard dose of 800mg pazopanib taken fasted. In addition we investigated the differences in GI-toxicity, patient satisfaction and patient's preference for either intake. The effect of the controlled intake with GAS on pazopanib exposure was performed as a sub-analysis of the DIET data.

Results / Conclusions

The intake of 600mg pazopanib with food resulted in a bioequivalent exposure compared to a standard pazopanib dose without food. No differences were seen in GI-toxicities under both intake regimens. Most of the patients preferred the intake with continental breakfast. The intake of pazopanib with food is a more patient friendly intake regimen and possibly reduces treatment costs.

Despite the controlled intake regime of ingesting pazopanib 1 hour after GAS, a clinically relevant interaction between pazopanib and GAS was shown. An additional sub-analysis showed that the difference in exposure was only seen in patients using omeprazole. In patients using pantoprazole no difference in exposure was observed. The lower exposure of pazopanib might have clinical consequences. Pazopanib concentrations should be monitored and a pharmacological intervention should take place in order to prevent reduced treatment benefit.

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Ligand-Based Design of Allosteric ROR γ t Inverse Agonists

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ROR γ t is a nuclear receptor that plays an important role in the differentiation of Th17 cells associated with the pathogenesis of autoimmune diseases.^{1,2} Inhibition of ROR γ t with small molecules in order to disrupt this pathway could therefore be beneficial, to diminish the inflammatory response.³ The majority of reported modulators target the orthosteric binding site in the ligand binding domain of ROR γ t (e.g. T0901317, Figure 1). However, recently a novel type of inverse agonist (MRL-871) was identified, acting at an alternative, allosteric pocket (Figure 1).⁴ Whereas orthosteric ligands often lead to issues associated with selectivity and mutation-induced resistance, molecules that target the allosteric site could circumvent these problems and are therefore extremely valuable in drug discovery.

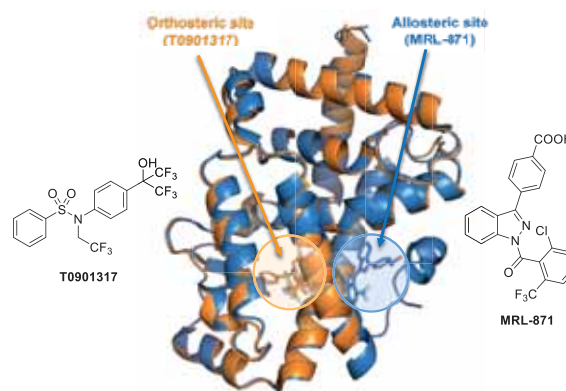


Figure 1. Orthosteric and allosteric ROR γ t ligand binding sites are shown by overlay of the crystal structures of ROR γ t LBD in complex with orthosteric inverse agonist **T0901317** (orange, PDB code: 4NB6) and allosteric inverse agonist **MRL-871** (blue, PDB code: 4YPQ).

Aims

Currently, the examples of allosteric ROR γ t ligands are limited to MRL-871 and some closely related indazoles. These compounds displayed highly promising in vivo activity but have been associated with poor pharmacokinetic profiles. In order to better exploit the strategy of allosteric modulation for therapeutic purposes, the aim is to identify novel chemotypes that effectively target the allosteric site of ROR γ t.

Methods

In silico pharmacophore screening and docking studies were used to discover a novel class of ROR γ t allosteric inverse agonists. These compounds were synthesized and evaluated in time-resolved FRET (TR-FRET) assays and Real-Time PCR. Structural information was obtained via X-Ray crystallography.

Results / Conclusions

The highest ranking hit structures from the pharmacophore search were all found to be based around the same trisubstituted isoxazole scaffold (Figure 2A). A small library around this scaffold was synthesized. Ultimately, lead compound **FM26** (Figure 2B) was found as the most active compound from the

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isoxazole series, showing a 36-fold improvement in potency compared to the initial isoxazole hit and clearly inhibiting transcriptional coactivator recruitment.⁵ The co-crystal structure of **FM26** in complex with the ROR γ t LBD was obtained (Figure 2C), proving allosteric binding via a similar mode as **MRL-871**. In addition, **FM26** was shown to significantly reduce IL-17a mRNA expression in EL4 cells, a marker of ROR γ t activity, and to have a promising ADME profile. This highlights the potential of this compound class, identified via a highly efficient ligand-based design approach, as allosteric ROR γ t modulators.

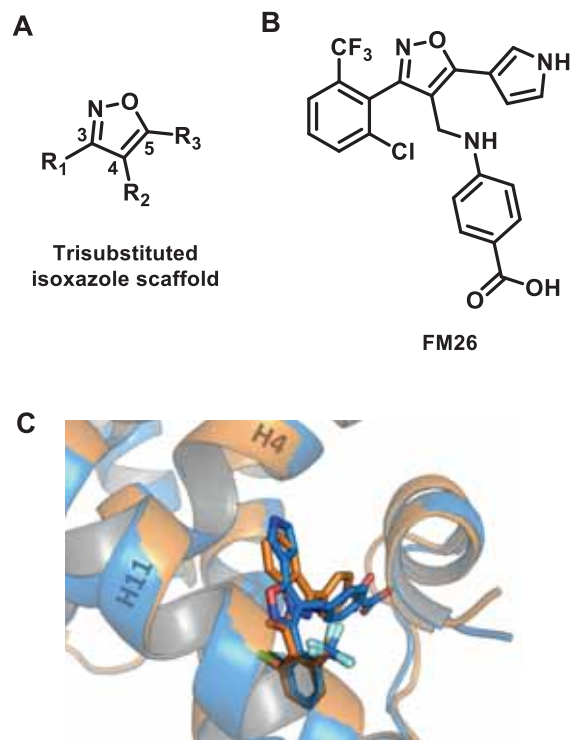


Figure 2. A) Trisubstituted isoxazole scaffold. B) Chemical structure of ligand FM26. C) Overlay of the crystal structure of ROR γ t bound to FM26 (blue, PDB code: 6SAL) and ROR γ t bound to MRL-871 (orange, PDB code: 4YPQ).

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The Interplay of Wnt- and PPAR γ -signaling in macrophages & vascular smooth muscle cells during vascular calcification

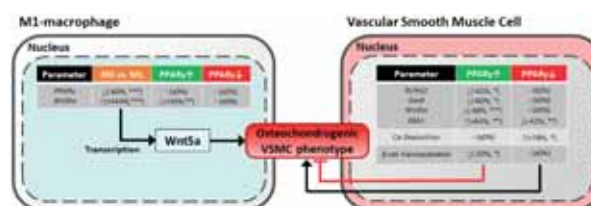
Aim

Due to the heterogeneity in terms of atherosclerotic plaque morphology and the participating cells, varying grades of vascular calcification occur in atherosclerosis. There is plausible evidence that sheet-like macrocalcifications have a stabilizing effect on the atherosclerotic plaque, whereas spotty- or microcalcifications are often associated with plaque instability and rupture, thus leading to a poor prognosis for patients. Macrophages and vascular smooth muscle cells (VSMC) are key players in this process by modulating inflammation and vascular calcification (VC) during atherosclerotic plaque development. M1-macrophages can induce calcification in VSMC by releasing mineralizing micro-vesicles and pro-inflammatory cytokines. Emerging evidences suggest a role for PPAR γ -signaling, which promotes adipogenesis, and Wnt-signaling, crucial for bone formation, in VC. The equilibrium of these pathways is disturbed in atherosclerosis, leading to an imbalance towards Wnt-signaling and subsequently to the calcification of atherosclerotic lesions. Pathological changes of the vascular environment can accelerate the expression of Wnt5a, a factor known to induce expression of osteo-chondrogenic genes in VSMC. In atherosclerotic lesions, Wnt5a was found co-localized with macrophages and inhibition reduced the expression of pro-inflammatory cytokines and the lesion size in *ApoE*^{-/-}-mice. Interestingly, VSMCs showed an upregulation of osteo-chondrogenic genes in lesions from PPAR γ deficient *LDLR*^{-/-}-mice. Contrarily, PPAR γ -stimulation leads to the secretion of Wnt5a-inhibitor Sfrp2 and other Wnt-Inhibitors (GSK3 β , Dkk-1) in several cell-types. We revealed a decreased PPAR γ expression in highly calcified lesions from patients of the BiKE-calc study. Furthermore, we observed a 2-fold upregulation of Wnt5a expression in primary macrophages treated with serum from patients with acute myocardial infarction. These supports our hypothesis that M1-macrophages can promote calcification of VSMC by secreting Wnt5a, which possibly can be inhibited by PPAR γ -signaling.

Results & Conclusion

PPAR γ -stimulation decreased the expression of osteo-chondrogenic genes (Sox9 -60%; Runx2 -62%, $P < 0.05$) in calcifying synthetic VSMC, but did not decrease calcium deposition. Wnt5a was decreased (-48%, $P < 0.001$), while Dkk-1 was increased (+64%, $P < 0.01$). In addition, ICC revealed a decreased nuclear translocation of active β -catenin (-20%; $P < 0.01$) and therefore a lower Wnt/ β -catenin signaling activity. PPAR γ -blockade did not change the expression of osteo-chondrogenic genes, but increased calcium-deposition (+78%, $P < 0.05$) and decreased expression of Dkk-1 (-42%; $P < 0.05$) in VSMCs. In M1 vs. M0-macrophages, PPAR γ expression was decreased (-60%, $P < 0.001$) while Wnt5a was increased (+444%, $P < 0.001$). PPAR γ -stimulation reduced Wnt5a expression (-45% $P < 0.01$) in M1-macrophages. Overall, the decreased PPAR γ -expression observed in human samples and in our in vitro study, may lead to the expression of osteogenic factors via the regulation of Wnt5a expression. PPAR γ -stimulation could be of clinical importance and deciphering the molecular interplay between PPAR γ and Wnt5a will help to demonstrate the relevance of this signaling for VC.

Graphical abstract



PhD Student Competition

Pharmacokinetic-pharmacodynamic target attainment of ciprofloxacin in adult patients on general wards with adequate and impaired renal function

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There are no prospective data on pharmacokinetic-pharmacodynamic (PK-PD) target attainment after the guideline-recommended dose reduction of the antibiotic ciprofloxacin in patients with impaired renal function (eGFR < 30 ml/min/1.73 m²).

Aims

This study aims to investigate AUC/MIC ≥ 125 in adult patients on general wards with adequate and impaired renal function receiving regular and reduced doses of ciprofloxacin.

Methods

In this prospective observational cohort study adult patients on general wards of a Dutch university hospital were included when treated with ciprofloxacin. Three blood samples per patient were prospectively obtained for ciprofloxacin concentration measurement in the first 48 hours of treatment, complemented with samples from waste material. For AUC calculation Bayesian analysis was performed using a population pharmacokinetic model developed by non-linear mixed effects modelling.

Results / Conclusions

A total of 40 patients were included, of which 8 patients with impaired renal function, all treated with a guideline-recommended reduced dose of ciprofloxacin. Using the clinical breakpoint MIC of most isolated bacteria (0.25 mg/L), the AUC₀₋₂₄/MIC ≥ 125 was attained in 38% of patients with adequate renal function receiving a regular dose and in 13% of patients with impaired renal function receiving a reduced dose (Figure 1). Median drug exposure in the first 24-hours of treatment (AUC₀₋₂₄) for patients with impaired renal function receiving a reduced dose was 17.9 mg/L*h, which was statistically significantly lower than the median AUC₀₋₂₄ for patients with adequate renal function receiving a regular dose (29.4 mg/L*h, $p < 0.01$). AUC₀₋₂₄/MIC ≥ 125 is not attained in the majority of adult patients on general wards for clinically relevant bacteria with MIC-values at or just below the clinical breakpoint.

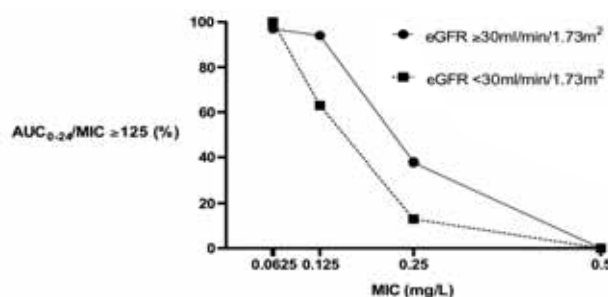


Figure 1. Calculated percentage of patients attaining the PK-PD target of AUC₀₋₂₄/MIC ≥ 125 at different MIC-values (0.0625, 0.125, 0.25, 0.5 mg/L), stratified per renal function group.

Searching for signs of asymmetry in histone inheritance

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Whether histone inheritance is truly symmetric and how new and old histones are deposited onto leading and lagging strands during replication are open questions. Through metabolic labeling of new histones and single strand sequencing of the resulting affinity-purified DNA we search for answers.

Aims

Chromatin is disrupted upon replication fork passage, and nucleosomes are rapidly reassembled on newly synthesized DNA through recycling of evicted parental histones and de novo deposition of new histones. Replication starts at thousands of sites during S-phase and replication fork directionality (RFD) and leading and lagging-strand replication therefore alternate along chromosomes. Potential biases in segregation of histones during replication will thus result in a specific pattern of sister chromatid asymmetry. We investigated the distribution of new histones on sister chromatids and linked this distribution to RFD to understand histone segregation.

Methods

We developed Double-Click-seq to track histone recycling and de novo deposition genome wide. We used metabolic labelling of both new histones and new DNA to track the deposition of new histones during replication. Human RPE-1 cells were labeled with the amino acid derivative azidohomoalanine (AHA) and the nucleoside analogue 5-ethynyl-2'-deoxyuridine (EdU). In two sequential click reactions, each coupled to streptavidin enrichment, new DNA coming from new histones was sequenced in a strand specific manner to score genome-wide sister chromatid histone partition. The effect of co-incubation with low-dose hydroxyurea (HU), as a model of replication stress, was also investigated.

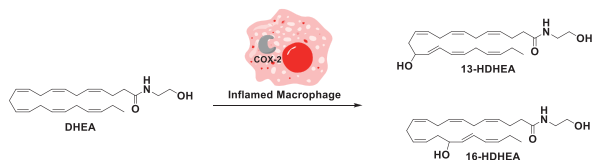
Results / Conclusions

Deposition of new histones is slightly biased towards the lagging strand. The process is reversed during replication stress.

ABS-67583495

Identification of novel enzymatic COX-2 products of N-acyl ethanolamine derived ω -3 poly-unsaturated fatty acids in inflamed macrophagesIan de Bus^{1,2}, Han Zuilhof¹, Renger Witkamp², Bauke Albada¹, Michiel Balvers²¹Laboratory of Organic Chemistry, Wageningen University & Research, Wageningen, The Netherlands ²Nutrition and Pharmacology Group, Division of Human Nutrition, Wageningen University & Research, Wageningen, The Netherlands

Cyclooxygenase-2 (COX-2) is a non-constitutional enzyme that is specifically expressed during inflammation. The enzyme oxygenates poly-unsaturated fatty acids (PUFAs) as well as neutral lipids like the endocannabinoid arachidonoyl ethanolamide (AEA), leading to the formation of regulators of inflammation (1). However, previous reports suggested that N-acyl ethanolamine derivatives of ω -3 PUFAs, like docosahexaenoylethanolamide (DHEA) could also interact with COX-2. For example, DHEA treatments resulted in decreased formation of pro-inflammatory regulators in inflamed macrophages (2). To better understand the interactions between the N-acyl ethanolamine derived PUFAs and COX-2, we designed a new cell free screening assay consisting of hCOX-2 incubations followed by LC-HRMS analysis to identify novel metabolites. We demonstrated the formation of several novel oxygenated metabolites of N-acyl ethanolamine derived PUFAs, of which 13-HDHEA and 16-HDHEA were shown to be the most important hCOX-2 products of DHEA. For the novel COX-2 metabolites we developed a UPLC-MS/MS quantification platform, which we used to quantify the product formation. Next, we quantified the novel metabolites 13-HDHEA and 16-HDHEA in our inflamed macrophage model. Control experiment with the selective COX-2 inhibitor celecoxib, proved that the metabolites were indeed COX-2 metabolites.



We identified novel enzymatic COX-2 products of N-acyl ethanolamine derived ω -3 poly-unsaturated fatty acids and developed an UPLC-MS/MS based quantification platform to quantify the novel metabolites. In addition, we showed that the DHEA derived metabolites, 13-HDHEA and 16-HDHEA, were produced in inflamed macrophages, suggesting a possible role in the resolution of inflammations.

Acknowledgements

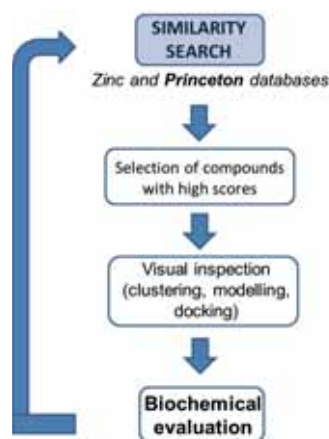
This research project is funded by the VLAG Graduate School of Wageningen University & Research

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ABS-67404210

Discovery of antitubercular inhibitors of DXS from the MEP pathway by ligand-based virtual screeningDi Zhu^{1,2}, Tiziana Masini², Céline Simonin³, Sandra Johannsen¹, Jörg Hauptenthal¹, Robin M. Gierse¹, Mahendra Awale³, Rita Nasti², Ramon van der Vlag², Jean-Louis Reymond³, Anna K. H. Hirsch^{1,2}¹Department for Drug Design and Optimization, Helmholtz Institute for Pharmaceutical Research (HIPS) – Helmholtz Centre for Infection Research (HZI), Saarbrücken, Germany;²Stratingh Institute for Chemistry, University of Groningen, Groningen, The Netherlands; ³Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland

Antimicrobial resistance has become a global health concern, urgently calling for the development of novel antibiotics. In this study, we carried out a ligand-based virtual screening campaign based on the structure of previously identified inhibitors¹ of the enzyme DXS from the methylerythritol phosphate (MEP) pathway. With the help of a 3D-shape and pharmacophore similarity algorithm², we identified three unprecedented scaffolds as slow, tightly binding inhibitors, targeting the catalytic center of Mycobacterium tuberculosis DXS, with K_i^* values of 40 – 1300 nM. All three classes of hits show remarkable antibacterial activity in cell-based assays against multi- and extensively drug-resistant strains of Mycobacterium tuberculosis. The most promising hits appear to be selective over related mammalian enzymes, and their favorable metabolic stability, lack of cytotoxicity against a human lung cell line and low frequency of resistance development make them excellent starting points for optimization to a new generation of selective and potent antitubercular agents with an unexploited mechanism of action.³

**Workflow of ligand-based virtual screening****References**

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ABS-66439724

Precise gene editing reveals role of FES tyrosine kinase in neutrophil phagocytosis via SYK activation

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Introduction

The successful development of new kinase-targeting drugs strongly depends on our understanding of underlying molecular and cellular mechanisms of action, i.e. preclinical target validation.¹ A key step in this process is to obtain proof of target engagement, which correlates the exposure at the site of action to a pharmacological and phenotypic readout. Information about kinase engagement is essential for determining the dose required for full target occupancy without inducing undesired off-target activity.²

Objectives

The aim of this study was to develop chemical tools that report on target engagement of endogenously expressed protein kinases by small molecules in human cells.

Methods

We describe a chemical genetics strategy that allows the study of non-receptor tyrosine kinase FES, a promising therapeutic target for cancer and immune disorders. CRISPR/Cas9-mediated gene editing was used in combination with a rationally designed, complementary fluorescent probe to visualize endogenous FES activity in HL-60 cells. We replaced a single oxygen atom by a sulphur in a serine residue at the DFG-1 position of the ATP-binding pocket in an endogenously expressed kinase, thereby sensitizing the engineered protein towards covalent labeling and inactivation by a fluorescent probe.

Results/Conclusions

Our results reveal that FES plays a key role in the phagocytosis of bacteria by activation of SYK kinase, a central regulator of immune function in neutrophils. Moreover, we demonstrate that our chemical genetics strategy is not limited to FES and can also be applied to multiple other kinases. The selectivity acquired by combining gene editing and a complementary probe brings the advantages of acute, pharmacological inhibition without the need for extensive hit optimization programs to identify compounds of adequate potency and selectivity. We thus envision that the presented methodology could provide powerful tools to study the function of poorly characterized kinases and aid in their validation as therapeutic targets.

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pH-responsive smart polymersomes for matrix metalloproteinase-1 delivery as a promising approach for the treatment of liver cirrhosis

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Introduction

Liver cirrhosis is a growing health problem affecting millions of people worldwide. Chronic liver injury leads to the formation of scar tissue due to the excessive accumulation of extracellular matrix, mainly collagen-I and -III produced by activated hepatic stellate cells (HSCs). Currently, there are no therapies available and liver transplantation is the only option, however has limitations¹. Matrix metalloproteinase-1 (MMP1) is an enzyme that degrades the scar tissue by degrading collagen-I and -III favoring liver regeneration².

Aims

We hypothesized that liver-specific delivery of MMP1 to degrade collagen as a promising approach for the treatment of liver cirrhosis. We studied the therapeutic efficacy of MMP1 on human HSCs. Using state-of-the-art technologies, we synthesized innovative pH-responsive smart MMP1-polymersomes with the MMP1 decorated on the polymersome surface³.

Methods

In-vitro studies were performed on TGFβ-activated human HSCs to evaluate the efficacy of MMP1 on the cell viability, gene and protein expression of collagen and HSCs activation marker αSMA. Polymersomes were fabricated using the pH switch method and MMP1 post-loading at pH 5-6 was performed to increase the interaction between MMP1 and the polymersome membrane. Physicochemical characterization and enzymatic assays were performed to characterize the attachment and functionalization of enzyme on polymersomes. Finally, cell viability assays using synthesized polymersomes were performed on HSCs.

Results/conclusions

MMP1 showed dose-dependent inhibition of collagen-I, -III and αSMA expression in TGFβ-activated human HSCs with no significant effects on cell viability. Decoration of MMP1 on the surface of the polymersome was successfully established with an affinity of 30%, without inhibition of function. Synthesized MMP1-polymersome hybrid structures showed favorable size (~180nm at pH 6 and ~140 nm at pH 8) and charge (positive at pH 6 and negative at pH 8). Polymersomes did not induce significant effects on cell viability at the tested doses from 0-16μg/mL. MMP1-polymersome hybrid structures will be tested for therapeutic efficacy on TGFβ-activated human HSCs and will be evaluated on CCl4-induced liver cirrhosis mouse model. In conclusion, we present an innovative approach of MMP1 delivery for the treatment of liver cirrhosis.

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ABS-64927083

Quantitative systems pharmacology modelling of Parkinson's disease for determining target suitabilitySuruchi Bakshi^{1,3}, Chao Chen², Piet H. van der Graaf^{1,3}¹Certara QSP, Breda, 4818 SJ, Netherlands and Canterbury, CT2 7FG, UK; ²Clinical Pharmacology Modelling & Simulation, GlaxoSmithKline, 1 Iron Bridge Road, UB11 1BT, Uxbridge, UK; ³Systems Biomedicine and Pharmacology, LACDR, Leiden University, P.O. Box 9502, 2300 RA Leiden, Netherlands.

Parkinson's disease (PD) is a progressive neurodegenerative disease with no disease-modifying therapies so far. Multiple factors such as ageing, environment and genetics contribute to neurodegeneration and dopamine deficiency in PD. Mathematical modelling can help understand such complex multifactorial neurological diseases (1,2).

Aims

We aim to determine suitability of various PD targets using quantitative systems pharmacology (QSP) approach. For this purpose, we have constructed a QSP model of PD with a focus on mechanistic molecular machinery responsible for PD pathogenesis. The model is extended to simulate spatial PD propagation through the brain through diffusion of extra-cellular Asyn species. Challenging this model using hypothetical monoclonal antibodies (mABs) against various targets is expected to give insight into target suitability.

Methods

The model is informed by previously-published models of Asyn aggregation and its feedback with oxidative stress (3,4). These models are integrated with each other and combined with novel elements of Asyn secretion and reuptake. Further, diffusion of misfolded Asyn through the brain is included. The combined ordinary and partial differential equation model of PD is coded using method of lines (5) and simulated with Matlab. The resultant model is verified using various clinical data and subjected to hypothetical monoclonal antibody drugs against selected targets to quantify disease attenuation.

Results / Conclusions

The naïve PD model (i.e. in absence of drugs) displays existence of two steady states – one with low Asyn misfolding and low oxidative stress – presumed to be the healthy state, and the other with high Asyn misfolding and high oxidative stress – presumed to be the diseased state. Switching of healthy to diseased state can occur due to excess misfolded Asyn accumulation. The model also predicts spatial PD propagation due to the diffusion and cellular uptake of Asyn. Antagonizing various potential drug targets results in differential level of disease attenuation, thus allowing us to rank targets in their order of suitability. This exercise is expected to inform drug discovery efforts.

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ABS-67651539

Multicyclic Peptides via Templated Tandem CLIPS/CuAAC CyclizationsG.J.J. Richelle^{1,2}, M. Schmidt^{1,3}, H. Hiemstra¹, T. Nuijens³, J.H. van Maarseveen¹, P. Timmerman^{1,2}¹Van 't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, The Netherlands, ²Pepscan Therapeutics, Lelystad, The Netherlands, ³Enzyperp B.V., Geleen, The Netherlands

Multicyclic peptides provide a very attractive molecular format for the design of novel therapeutics.^[1] Therefore, novel routes for synthesis and HTS-screening of this fascinating class of compounds are desperately needed. A decade ago, we launched a novel scaffold-assisted peptide-cyclization technology platform, termed "CLIPS", to generate in a one-steps procedure a new class of mono- and bicyclic peptides able to act as potent inhibitors of hitherto undruggable therapeutics targets.^[2,3]

Following this, we now present a next-generation technology that combines both CLIPS and CuAAC chemistry into a one-pot methodology that enables the manufacturing of structurally complex peptide multicycles (tri-,^[4] tetra-,^[5] penta- and hexacyclic constructs) by using either linear or backbone-cyclized^[6] peptides (see Figure). We present the chemical synthesis of two different types of CLIPS/CuAAC-scaffolds (**T4** and **T6**) and show how these scaffolds behave in the synthesis of highly complex peptide constructs. We further illustrate the broad applicability of the technology via a successful first screening for tricyclic CLIPS/CuAAC peptides as novel antimicrobials.

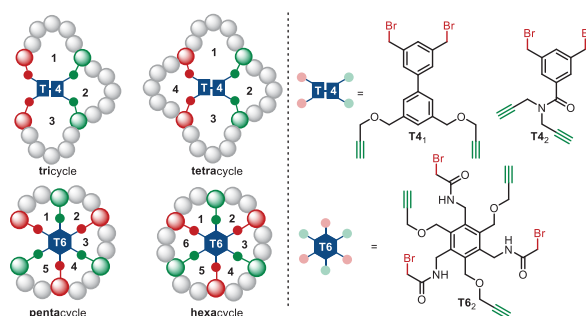


Figure. Novel multicyclic peptide topologies by constraining of linear- and backbone cyclized peptides onto T4- and T6 scaffolds.

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Innovative monitoring...

ABS-66382414

Effects of regulatory major objections on reimbursement decisions in Europe

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To enhance timely access to medicines, the European Medicines Agency (EMA) may accept less conclusive clinical evidence with a higher level of uncertainty at time of marketing authorization (MA). How this affects reimbursement recommendations remains unknown.

Aims

We aimed to (i) determine whether the existence of major clinical objections (MOs) regarding phase III data during the MA procedure as a measure for uncertainty is associated with negative reimbursement recommendations, and (ii) identify differences in clinical studies used for decision-making by EMA and reimbursement agencies.

Methods

For a cohort of innovative medicines that received MA (2009 & 2010, excluding vaccines) we identified all publicly available initial reimbursement recommendations in England, France, the Netherlands and Scotland. Risk ratios (RRs) and 95% confidence intervals (CI) were calculated overall and per jurisdiction for the association between MOs identified in MA dossiers (Putzeist et al, 2012) and negative reimbursement recommendations. Data on clinical studies were extracted from reimbursement dossiers and EMA's public assessment reports (EPARs) and compared.

Results

For 35 medicines, 109 reimbursement recommendations were made, of which 43 percent were negative. The presence of clinical MOs was associated with an increased, although non-significant, risk for a negative reimbursement recommendation: 1.4 (95% CI 0.9 to 2.3). For England, France, the Netherlands and Scotland, the RRs were 0.6, 1.5, 2.1 and 1.1, respectively (all non-significant). The proportion of studies in the EPAR also used for reimbursement recommendations varied from 24 (England) to 55 (France) percent. The proportion of studies used for reimbursement decision-making that were not included in the EPAR varied from 13 (France) to 55 (England) percent.

Conclusions

Acceptance of uncertainties by regulators may be associated with negative reimbursement recommendations. This study suggests different considerations regarding clinical evidence between regulators and reimbursement authorities. Many studies in the EPAR are not used for reimbursement recommendations, and vice versa.

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Therapeutic Use...

ABS-68356840

POPular Genetics - A Genotype-Guided Strategy for Oral P2Y₁₂ Inhibitors in Primary PCI

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It is unknown whether patients undergoing primary percutaneous coronary intervention (PCI) benefit from genotype-guided selection of oral P2Y₁₂ inhibitors. Therefore we conducted the POPular-Genetics study.

Aims

To determine whether a CYP2C19 genotype-guided strategy for selection of oral P2Y₁₂ inhibitors can reduce bleeding risk without increasing thrombotic risk in patients with STEMI undergoing primary PCI with stent implantation.

Methods

We conducted a 1:1 randomized, open-label, assessor-blinded trial in which patients undergoing primary PCI with stent implantation received either a P2Y₁₂ inhibitor based on early CYP2C19 genetic testing or standard treatment with either ticagrelor or prasugrel for 12 months. Carriers of CYP2C19*2 or CYP2C19*3 loss-of-function alleles received ticagrelor or prasugrel, while noncarriers received clopidogrel. The two primary outcomes were net adverse clinical events, defined as all-cause death, myocardial infarction, definite stent thrombosis, stroke and Platelet Inhibition and Patient Outcomes (PLATO) major bleeding (tested for noninferiority, with a noninferiority margin of 2% for the absolute difference) and combined PLATO major and minor bleeding at 12 months.

Results / Conclusions

For the primary analysis, 2488 patients were included; 1242 in the genotype-guided group and 1246 in the standard-treatment



Therapeutic Use...

group. The primary outcome occurred in 63 patients (5.1%) in the genotype-guided group and 73 patients (5.9%) in the standard-treatment group (absolute difference -0.7%; 95% confidence interval [CI], -2.0% to 0.7%; P for noninferiority 0.0002). The co-primary bleeding outcome occurred in 122 patients (9.8%) in the genotype-guided group and in 156 patients (12.5%) in the standard-treatment group (hazard ratio, 0.78; 95% CI, 0.61 to 0.98; P=0.04).

In patients undergoing primary PCI, a CYP2C19 genotype-guided strategy for selection of oral P2Y₁₂ inhibitor therapy was noninferior to standard treatment with ticagrelor or prasugrel at 12 months with respect to thrombotic events while lowering bleeding rates. (Netherlands Trial Register number NL2872, ClinicalTrials.gov number NCT01761786).

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Development of prescribing knowledge and skills of junior doctors in the first year after graduation

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Poor prescribing has negative effects on patient safety and healthcare costs. Medical students in Europe lack prescribing knowledge and skills at graduation (Brinkman et al., 2017, Brinkman et al., 2018), probably due to inadequate education in clinical pharmacology and therapeutics during the undergraduate medical curriculum (Brinkman et al., 2017). However, little is known about the development of prescribing knowledge and skills of junior doctors after graduation. This is important to develop relevant educational interventions after graduation.

Aims

Investigating how the prescribing knowledge of junior doctors in the Netherlands and Flanders develops in the first year after graduation.

Methods

This is a prospective cohort study among medical graduates from 11 medical schools in the Netherlands and Flanders. In total, 1.584 medical students graduating between July 2016 and March 2018 were invited to participate. They were asked to complete an online assessment at three different time points: T0= around graduation, T1= six months after graduation, T2= one year after graduation. Each assessment contained of 35 multiple choice questions extracted from the Dutch Pharmacotherapy Assessment database. The primary outcome is the total percentage of correctly answered questions.

Results / Conclusions

In total, 556 (35%) medical students agreed to participate of which 326 (58%) completed all three rounds. Overall,

prescribing knowledge increased significantly from 69% (SD 13,0) to 77% (SD 11,4) in the first six months and then decreased to 71% (SD 13,8) at the end of the year (p<0.001). For 5/7 topics (anticoagulants, cardiovascular drugs, antidiabetics, psychotropics and basic pharmacokinetics and drugs calculation) there was a significant increase in prescribing knowledge during the first six months (p<0.001). However, except for basic pharmacokinetics and drugs calculation, this knowledge decreased during the second six months (p<0.001). For both antibiotics and analgesics, the prescribing knowledge increased significantly after one year (both p<0.001).

There is an increase in prescribing knowledge of junior doctors in the first six months after graduation but then, except for antibiotics and analgesics, declines in the ensuing six months. This study indicates that educational interventions after graduation are needed to improve prescribing knowledge.

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ABS-66446288

Trends in Blood Pressure Thresholds for Initiating Treatment in Patients with Type 2 Diabetes in Primary Care

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Introduction

There have been changes over time in the recommended systolic blood pressure (SBP) threshold for initiating blood pressure (BP) lowering treatment in patients with type 2 diabetes (T2D). Particularly, attention for a more personalized approach has increased, including less strict thresholds for the elderly and frail population.

Objectives

To assess trends in SBP thresholds at initiation of BP lowering treatment in T2D patients and the influence of patients' age and frailty on these thresholds.

Methods

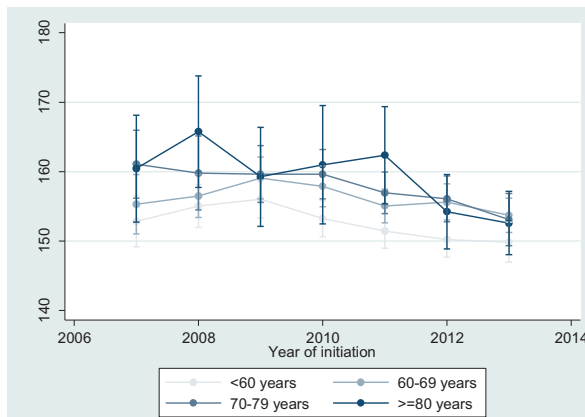
We used the Groningen Initiative to Analyse Type-2 diabetes Treatment (GIANTT) database, which includes data about patients with T2D treated in primary care in the north of the Netherlands. For each calendar year between 2007 and 2013, patients were included if they had initiated BP lowering treatment, and had an SBP measurement within 120 days before or at initiation. The influence of year, age (< 60, 60 – 69, 70 – 79 and ≥ 80 years old) or frailty (frailty index, tertiles) and the interaction between year

Therapeutic Use...

and age or frailty on SBP value at initiation were assessed using multilevel regression analyses, adjusted for confounders.

Results

A total of 4,170 patients initiating BP lowering treatment in the period 2007 to 2013 were included (47% female; 68% younger than 70 years, 22% aged 70 to 79 and 11% 80 years or older). The average SBP level at treatment initiation changed significantly over time, from 155 mmHg (SD 22 mmHg) in 2007, rising to 158 mmHg (SD 21 mmHg) in 2009 and then decreasing to 152 mmHg (SD 22 mmHg) in 2013. This quadratic trend was statistically significant ($p < 0.000$). In general, patients 70 years and older initiated treatment at higher BP thresholds than younger patients but similar decreasing trends after 2009 were observed for all age groups (figure). There were no statistically significant differences in BP thresholds between patients with different levels of frailty over the years. The relationship between calendar year and BP threshold was not influenced by age or frailty.



Conclusions

After an initial rise, the observed BP thresholds at initiation of BP lowering treatment decreased over time and these trends were not influenced by age or frailty. These similar trends over time are in contrast with guideline recommendations advocating less strict BP thresholds after 2011 for older and frail patients.

Late breaking science

ABS-66439630

Targeting mitochondria to prevent ferroptosis: A focus on cAMP signaling

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Since their discovery, exchange proteins directly activated by cAMP (EPAC) proteins, have been implicated in a wide range of cellular functions, including oxidative stress and cell survival. Mitochondrial-dependent oxidative stress has been associated with the progressive neuronal death underlying the pathology of many neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. In many cases, these cellular functions have been shown to be coordinated by scaffolding proteins known as A-kinase anchoring proteins (AKAPs). One AKAP family member, mitochondrial AKAP1 is associated with EPAC proteins and it contributes to oxidative stress responses by preventing cell death via mitochondrial dynamics.

Aims

In this study, the cellular distribution of EPAC proteins in neuronal cells i.e. immortalized hippocampal (HT22) cells was investigated and in addition the differential expression in the mitochondrial fraction was also investigated. Furthermore, the role of EPAC proteins and AKAP1 in oxidative stress were investigated by pharmacological targeting of EPAC in HT22 cells, and over expression of AKAP1 respectively. Finally, mitochondrial respiratory chain activity in the presence of various EPAC modulators was also examined in HT22 cells.

Methods

mRNA and protein expression of EPAC in HT22 cells was determined by qPCR and western blot analysis respectively. One type of cell death was represented by erastin-induced ferroptosis, in which the oxidative stress was initiated by a reduction of glutathione levels and a dysfunction of the iron metabolism. Mitochondrial respiratory chain activity was determined by high-resolution respirometry.

Results/Conclusions

It was showed upon specific pharmacological modulation of the two forms of EPAC proteins, EPAC1 and EPAC2, that these isoforms exert opposing effects on a diverse subset of oxidative stress. EPAC1 inhibition prevented cell stress linked to glutathione loss, while EPAC2 inhibition had limited effects during impairment of cellular thioreductase enzymes. These results support previous findings demonstrating that overexpression of AKAP1 offers protection against oxidative stress. Taken together, this data indicates that EPAC1 and EPAC2 have different cellular localization and functions within the cells and may thus be involved in different oxidative stress pathways.

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Late breaking science

ABS-65997846

Photoswitchable ligands as tools for dynamic modulation of histamine receptors

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Histamine receptors are widely distributed throughout the body and respond (in a paracrine/autocrine manner) to local increases in histamine. Hence, it would be therapeutically advantageous to only locally influence histamine receptors rather than systemic targeting of the receptor to avoid unwanted side effects. Temporal and spatial control of drug effects can be induced utilizing photoswitchable ligands. Photoswitchable ligands can reversibly photo-isomerize from the trans to the cis isomer upon illumination with specific wavelengths. The resulting conformational change in the ligand may result in distinct changes in binding affinity and/or intrinsic activity of the isomer for the receptor. To this aid, we have developed and characterized a toolbox of photoswitchable ligands for the different histamine receptor subtypes that enables dynamic optical control of their activity with high temporal resolution. Moreover, the option to locally modulate the receptor activity with light opens opportunities to investigate the local role of histamine receptor signaling in vivo.

ABS-66249706

Exploring the potential of immune modulation triggered by nanobody-targeted photodynamic therapy

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Introduction

Photodynamic therapy (PDT) is an anti-cancer approach used in the clinic which relies on the local excitation of a photosensitizer by light and subsequent transfer of energy to molecular oxygen, leading to the formation of reactive oxygen species and direct cell death. Nanobody-targeted photodynamic therapy (NB-PDT) has been developed as a potent and more tumor-specific approach compared to conventional PDT [1]. Interestingly, conventional PDT is able to induce immunogenic cell death, characterized by the exposure/release of damage associated molecular patterns (DAMPs) from dying cells, which can lead to anti-tumor immune responses and complete tumor eradication [2].

Aim

We aim at understanding the possible immune modulation triggered by NB-PDT.

Methods

The photosensitizer IRDye700DX was conjugated to the EGFR-targeted NB 7D12 and used to perform NB-PDT on EGFR-overexpressing A431 tumor cells. After 30 min incubation with the NB-PS conjugates, A431 cells were exposed to a 690nm laser with a light dose of 10 J/cm². Cellular localization of the DAMPs HSP70 and HMGB1 was visualized on treated cells by confocal microscopy. Release of HSP70, ATP and inflammatory cytokines (i.e. IL-1 β , IL-6 and IL-8) were quantified in the supernatants of treated tumor cells. Furthermore, human monocyte derived dendritic cells (moDC) were generated and co-incubated with

treated tumor supernatants. Expression on moDC of maturation markers CD86 and MHCII was analyzed by flow cytometry.

Results

The cytoplasmic DAMP HSP70 was detected on the cell membrane after mild NB-PDT (1 nM conjugate), while it was detected in the medium after highly cytotoxic NB-PDT (25 nM conjugate). The nuclear DAMP HMGB1 was found in the cell cytoplasm under both NB-PDT conditions. Furthermore, cells treated with highly cytotoxic NB-PDT showed an increased release of ATP and pro-inflammatory cytokines IL-1 β and IL-6, and a decreased release of pro-tumoral IL-8. Lastly, supernatants collected from tumor cells treated with highly cytotoxic NB-PDT were able to induce the phenotypic maturation of human moDC, as indicated by the upregulation of CD86 and MHC II on the cell surface.

Conclusion

These results are the first to suggest immune modulation by NB-PDT, which can be exploited to increase NB-PDT efficacy even further.

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Acknowledgements

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ABS-66327711

Lyp-1 Liposomes Encapsulating a Liver X Receptor Agonist Target to Atherosclerotic Plaques and Reduce Macrophage Content

Aims

Atherosclerosis is the predominant underlying pathology of cardiovascular disease and is one of the leading causes of death worldwide. It is characterized by the retention of lipids such as cholesterol in macrophages (foam cells) in the intima of arteries [1]. Liver X receptor (LXR) agonists, such as GW3965, are promising compounds for treating atherosclerosis since they induce reverse cholesterol transport in foam cells. However, as accumulation of LXR agonists in the liver can lead to high levels of lipids in the plasma or liver [2]. We, therefore, encapsulated GW3965 in liposomes to attempt to reduce the side effects, and functionalized these liposomes with a peptide Lyp-1 (CGNKRTRGC) [3] to target the drug to atherosclerotic lesions and enhance their therapeutic effectiveness.

Methods

Lyp-1 was synthesized and conjugated to PEG2000-DSPE. Synthesis was confirmed by LC-MS. Liposomes composed of DOPC:DOPS:DSPE-PEG:DSPE-PEG-Lyp-1:DOPC-Cy5 in mol% 75.9:19.4:3.0:7.0:1 were prepared using the dehydration-rehydration method. GW3965 was loaded in the bilayer of the liposomes. Liposomes were extruded to a size of around 80 nm (PDI < 0.1), and the zeta-potential of liposomes was around -20 mV as measured by DLS. Lyp-1 and drug content were measured by UPL. Drug loading was around 80%. For in vitro experiments, macrophages were cultured from bone marrow of LDLr^{-/-} mice. Foam cells were made by incubating these macrophages with oxLDL. Liposome uptake was measured by flow cytometry. In vivo targeting experiments were carried out in male LDLr^{-/-} mice on western type diet (WTD) for 13 weeks. Liposomes were injected i.v. and organs were harvested after 3 hours. Detection of fluorescent label in organs was performed using an in vivo imaging system, and specific uptake of liposomes was measured by flow cytometry. The effect of treatment with

Late breaking science

GW3965-loaded Lyp-1 liposomes or controls on atherosclerotic plaque development was performed in male LDLr^{-/-} mice on WTD for 8 weeks. Mice subsequently received i.v. injections twice per week for 5 weeks while maintaining WTD. GW3965 dose was 0.2 mg/mouse, and Lyp-1 dose was 35 µg/mouse. Mice were sacrificed and lipid analyses were performed on the plasma and livers. Atherosclerotic burden was assessed in the heart of mice by oil-red-O staining (lipid content) and MOMA2 staining (macrophage content).

Results/Conclusion

Lyp-1 liposomes specifically targeted to foam cells in vitro, showing 15x more uptake in foam cells compared to macrophages. While a large percentage of liposomes was taken up in the livers, kidneys and spleens of atherosclerotic mice, the Lyp-1-conjugated liposomes showed significant retention in the aortas, which was confirmed to be due to macrophage uptake. Plaque size was unchanged for all groups, but Lyp-1 liposomes significantly reduced macrophage content compared to PBS, free drug, and empty liposomes. None of the groups led to changes in plasma or hepatic lipid content, while lipogenic genes were significantly upregulated in the livers of mice receiving drug-containing treatments compared to PBS control, indicating that the liposomes do not fully protect against lipogenesis. In conclusion, Lyp-1 liposomes successfully target foam cells in atherosclerotic plaques for the delivery of GW3965, and decrease plaque macrophage content compared to free drug by almost 50%.

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ABS-66425312

Clinical implication of assessing quantitative plasma concentrations of antihypertensive drugs

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Objective

Therapeutic drug monitoring (TDM) is an accurate and reliable method to identify non-adherence to antihypertensive drugs. Although qualitative data are most important to determine non-adherence, quantitative data provide more insight into the dose at intake or time of intake. Therefore, the variance in quantitative values between patients derived by TDM, for eight antihypertensive drugs and four of their metabolites was studied.

Methods

In a previous study a TDM method for eight antihypertensive drugs and four of their metabolites (table 1) was validated in patients assumed to be adherent. The present data was retrieved from this validation study. Drug concentrations derived from a venipuncture were plotted for each measured drug in a C_{plasma} vs time graph. A Generalized Estimating Equation (GEE) was used to assess the influence of covariates including gender, dose, age, weight and the time interval between drug intake and sampling, on the C_{plasma}.

Results

A total of 135 patients (mean age of 59 ± 12 years, 59% male) were included. A high variability in C_{plasma} between patients was observed, especially at peak concentrations (table 1). The time interval between drug intake and sampling was of direct influence on the C_{plasma} of all drugs with the exception of hydrochlorothiazide and nifedipine. The drug dosage was only of influence on the C_{plasma} of amlodipine, losartan-carboxylic acid, canrenone and valsartan (p<0,05). No direct correlation (p>0.05) was found between drug levels and weight or age of the patient. Only for canrenone, a significant difference in C_{plasma} was found between males and females after correction for all other covariates (B-value -29.7, p=0.03).

Table 1 Fold-change in plasma concentrations at tmax of antihypertensive drugs with a same dosage.

Drug [metabolite]	Dose of drug to measure fold-change (mg)	Fold-change plasma concentration at supposed Tmax* (mcg/L)			Supposed Tmax (hours)
		lowest concentration	highest concentration	Fold - change	
Amlodipine	10	11.0	46.1	4.2	6-12
Enalapril	NA**	NA	NA	NA**	1
[Enalaprilate]	20	51.4	139.6	2.7	4
Hydrochlorothiazide	12.5	39.6	105.3	2.7	4
Losartan	NA**	NA	NA	NA**	1
[Losartan-ca]	100	18.1	637.0	35.2	4
Nifedipine	60	20.9	89.8	4.3	1.6-4.2
Perindopril	NA**	NA	NA	NA**	1
[Perindoprilate]	8	4.7	45.3	9.6	3-4
Spirolactone	12.5	14.0	188.6	13.5	2.6
[Canrenone]	25	42.0	98.0	2.3	4.3
Valsartan	320	1310.9	6876.4	5.3	2-4

NA = not available, losartan-ca = losartan-carboxylic acid, [] = metabolite
* Fold-change plasma was calculated with data from approximately 0.5 hours around the supposed Tmax if no range was supposed.

** Few samples around 1 hour after intake

Conclusion

The quantitative values of C_{plasma} of the eight antihypertensive drugs and their metabolites show a large inter-individual difference. Quantitative values cannot be used to determine the time of drug intake or the dose of a drug. The time of intake is related to the height of the C_{plasma}, as expected, but the influence of dose, weight, age and gender on drug levels differ largely between the measured drugs.

ABS-66281500

Sex proportionality in pre-clinical and clinical trials evaluated in the drug application dossiers

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Introduction

Concerns have been raised about an underrepresentation of women in drug trials and a lack of sex-specific analyses on drug responses. These concerns could imply that women benefit less from drug treatment or that women are at higher risk of adverse events when a drug is marketed.



Late breaking science

Aims

The aim of our study was to assess to what extent women were proportionally to disease prevalence included in all phases of drug development, and data presented in the dossiers supporting marketing approval of drugs. We additionally assessed whether there were sex differences in efficacy and safety of the evaluated drugs.

Methods

Data were extracted from drug application dossiers submitted for marketing authorization to the Dutch Medicines Evaluation Board. Preclinical animal studies, phase 3 studies, and pharmacokinetic studies of drugs approved for marketing authorization between 2011-2015 for the treatment of hepatitis C, HIV, depression, schizophrenia, epilepsy, heart failure, thrombosis, hypercholesterolemia, and diabetes were evaluated. Descriptive statistics and Pearson Chi Squared tests were used to assess respectively gender proportionality, efficacy and safety outcomes across trials.

Results

A total of 24 dossiers were evaluated. In all phases women were included, the mean proportion ranging from 30% in phase 1 trials to 42% in phase 3 trials. Of the animal pharmacodynamic studies, only 8% included either female or both male and female animals. However, an equal representation of male and female animals was present in the pivotal safety studies. When compared to disease prevalence, disproportionality in phase 3 trials was in favor of women for thrombosis and hepatitis C and in favor of men in schizophrenia, heart failure and hypercholesterolemia. Subgroup analyses for efficacy and sex-specific information on safety were present in all dossiers, and all drugs demonstrated efficacy in both men and women. Adverse events were more frequent in women than in men for both the treatment and the placebo groups.

Conclusion

Women are included throughout all phases of clinical drug research and sex-specific information on pharmacokinetics, efficacy, and safety are available in the dossiers. This reassures the external validity with respect to sex distribution of drugs at the time of app

Innovative gene targeting...

The use of CRISPR/Cas in clinical trials.

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FIGON, September 2019

Abstract

ATMPs (Advanced Therapy Medicinal Products) comprises cell therapies, gene therapeutics and tissue engineered products. Gene therapies include treatment with small oligonucleotides, gene-modified cells and DNA or viruses containing genes.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated nuclease 9 (Cas9), as a powerful genome-editing tool, has revolutionized genetic engineering. CRISPR/Cas9 has been used for the identification of potential molecular targets for cancer therapy. In addition, CRISPR/Cas9 and other gene editing techniques are being developed for treatment of patients with AIDS, cancer and other diseases.

Gene editing is not only a promising new branch treatment for severe diseases but may also be used in sports medicine. This raises the issue whether these techniques might be abused in the field of elite sports. Both the World Anti-Doping Agency (WADA) and the International Olympic Committee (IOC) have expressed concerns about this possibility. As a result, the method of gene doping has been included in the list of prohibited classes of substances and prohibited methods.

Current detection methods are insufficient to detect gene doping because they are limited to the detection of foreign drugs or proteins and unable to measure small changes in native human protein levels. New detection methods based on sequencing are the most promising preventive methods to counteract the possible application of gene doping.

Antibodies blocking CGRP or its receptor in the prophylactic treatment of migraine

E. Rubio-Beltran & A. Maassen van den Brink

Migraine is the most prevalent neurological disorder worldwide and has an immense socioeconomic impact. Whereas preventative treatment options for migraine previously only included drugs developed for diseases other than migraine such as hypertension, depression and epilepsy, now blocking calcitonin gene-related peptide (CGRP) has emerged as a mechanism for prevention of migraine attacks. CGRP has been shown to be released during migraine attacks and it may play a causative role in induction of migraine attacks. Apart from small molecule CGRP receptor antagonists (gepants), monoclonal antibodies, targeting either CGRP or the CGRP receptor have recently been developed and approved by the FDA/EMA. The antibodies are effective in the prophylactic treatment of migraine and are well tolerated. However, CGRP and its receptor are abundantly present in both the vasculature and in the peripheral and central nervous system, and are involved in several physiological processes. Therefore, blocking CGRP may pose a risk in subjects with comorbidities such as cardiovascular diseases. In addition, long-term effects are still unknown. The pros and cons of blocking CGRP or its receptor in migraine patients will be discussed during the lecture.

ABS-66418979

A systematic imaging-based screen to identify the microRNA regulatory landscape of the Nrf2 pathway

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Introduction

MicroRNAs are small non-coding RNAs. They regulate gene expression at the post-transcriptional level and are involved in many biological processes. Recent studies indicate that circulating microRNAs are stable and can be assessed in a non-invasive manner, making microRNAs interesting biomarker candidates. Furthermore, microRNA expression changes are linked to various diseases like cancer and neurodegenerative disorders. Moreover, research considering microRNAs showed an increasing importance of these small noncoding RNAs in different cellular stress response pathways, including e.g. oxidative stress, unfolded protein, and DNA damage response. So far, a systematic analysis of the functional role of all known microRNAs on individual stress response pathways is lacking.

Objective

Main objective is to determine the effect of microRNAs on the Nuclear factor-erythroid-2-related factor 2 (Nrf2) pathway (oxidative stress response).

Method

To monitor the Nrf2 pathway, different stress response reporter cell lines were used e.g. HepG2-Nrf2-GFP and HepG2-Srxn1-GFP, a direct downstream target of Nrf2. CDDO-Me (30 nM) was used to activate the Nrf2 pathway. MicroRNA mimics (2600) were used to enhance the effect of the endogenous microRNAs in order to detect their possible function in the Nrf2 pathway. Single cell live confocal imaging was used to measure the Nrf2-response after treatment. HepG2-Chop-GFP and HepG2-P21-GFP cells were used to investigate the effect of defined microRNA hits on the unfolded protein response and DNA damage response respectively. Whole transcriptome analysis was performed on HepG2 wild-type cells transfected with microRNAs or siRNAs to obtain information at gene level.

Results/Conclusion

We identified a set of 26 microRNAs able to alter the expression of the Nrf2 pathway. miR-1293 and miR-6499-3p were found to be the strongest inducers, while miR-200a-5p and miR-502-5p were found to be the strongest inhibitors of the Nrf2 pathway. Gene expression correlated with their protein products. Moreover, most Nrf2-enhancing microRNAs were also found to enhance Chop induction. Interestingly, the identified 26 microRNAs are known to play a role in cancer and neurodegenerative disorders like Alzheimer's disease.



Innovative gene targeting...

ABS-67602875

Nanobodies targeting and modulating G protein-coupled receptors outside in and inside out.

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G protein-coupled receptors (GPCRs) belong to the largest class of membrane proteins. Their prominent role in many (patho) physiological processes make GPCRs important drug targets. While many approved drugs act on GPCRs, only a small fraction of all 850 family members are currently being targeted, leaving an enormous potential for drug development.

Aims

Nanobodies have appeared to be ideal ligands to study, modulate, and exploit GPCRs. Their unique structure allows the binding to non-linear, conformational epitopes, such as those formed by the intra- or extracellular loops of GPCRs. Their small size also aids phage-library generation and phage expression. Here, we show examples of nanobodies targeting intra- or extracellular parts of chemokine receptors and their utilization in GPCR research and therapy.

Methods

Llamas were immunized with DNA encoding for the human chemokine receptors CXCR4, CXCR7 or the viral chemokine receptor US28, followed by boost immunizations with either cells or lipoparticles. VHH-phage libraries were constructed and used for three rounds of panning.

Results / Conclusions

For all three receptors, in outside-binding nanobodies antagonized ligand binding. Moreover, as bivalent constructs, the nanobodies displayed inverse agonistic activities on constitutively active receptors. Furthermore, intrabodies targeting US28 recognized and stabilized different receptor conformations and could completely block G protein signaling. The best binding nanobodies were further functionalized via the addition of different effector domains. Nanobody-Fc constructs induced antibody-dependent cellular cytotoxicity (ADCC) of CXCR4-overexpressing leukemic cells. Nanobody-photosensitizer conjugates allowed the specific killing of CXCR4-overexpressing HEK293 cells or US28 expressing glioblastoma cells via photodynamic therapy. These studies show the feasibility of developing nanobodies targeting GPCRs and illustrate their potential in fundamental GPCR research and as targeting moieties for diverse therapeutic conjugates.

Small Molecules

ABS-67452725

Targeting NLRP3 inflammasome: development of new covalent and non-covalent inhibitors of ATPase activity

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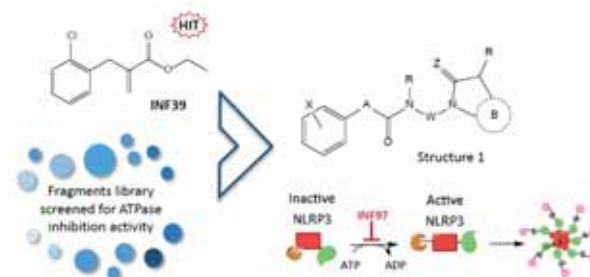
NLRP3 inflammasome is a multiprotein complex playing a key role in the intracellular activation of the innate immune system through activating cleavage of pro-inflammatory interleukins (IL)-1 β , IL-18 and triggering of pyroptotic cell death¹. In the last decades, several studies have highlighted the pivotal role of inflammasomes in the molecular control of inflammatory processes and the pathological role of NLRP3 inflammasome has been well established in different pathological settings.

Aims

The discovery of agents able to prevent inflammasome activation is a promising therapeutic strategy to decrease chronic inflammation and associated damage.

Methods

NLRP3 and few others inflammasome possess ATP-binding potential and intrinsic ATPase activity. Mutations in ATP-binding regions abolished their ATP-binding and ATPase activities and thereby resulted in impaired IL-1 β maturation. From a screening of different molecular fragments on recombinant human (rh) NLRP3 protein, a benzo[d]imidazol-1-one sub-moiety was identified as a weak inhibitor of ATPase activity. The fragment was functionalized using other structural motifs present in derivative INF39, previously identified as able to bind to NLRP3 and to hamper ATPase activity². Modulation of selected molecular moieties (Structure 1: A; B; X; W; Z; R1, R2) was performed using classical medicinal chemistry techniques such as bioisosteric replacement and refining of conformational flexibility.



Results / Conclusions

Newly synthesized compounds showed 5-10-fold improved NLRP3 ATPase inhibition with respect to starting fragments. INF97 elicits an interesting concentration-dependent ATPase inhibition (IC₅₀: 17.2 μ M, 15.4 – 19.2 C.L. 95%) and inhibits LPS/ATP triggered pyroptosis. Using a fragment-based approach, new NLRP3 ATPase inhibitors were obtained, and a new non-covalent hit compound (INF97) has been identified and characterized.

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Biologicals & Small Molecules

ABS-67614542

The role of 14-3-3 mediated regulation of the cancerous inhibitor of protein phosphatase 2a (CIP2A) in breast cancer cells

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The small molecule fusicoccin (FC) is able to stabilize protein-protein interactions (PPIs) between the regulatory proteins 14-3-3 and a subset of their client proteins. Stabilization occurs at the extreme C-terminus of the client protein which interacts with 14-3-3 based on a phosphorylated serine/threonine type III motif (xxx-pS/pT-(VLI)-COOH). It has previously been demonstrated that FC has anti-proliferative effects in various tumour cell lines. Here we show that the triple negative breast cancer cell line MDA-MB-468 is FC sensitive. Analysing 14-3-3 interacting proteins from MDA-MB-468 cell lysates using FC coupled to magnetic beads, we have identified the cancerous inhibitor of protein phosphatase 2a (CIP2a) as a putative 14-3-3/FC target. The oncoprotein CIP2a has been shown to play a crucial role in breast cancer affecting cell proliferation by inhibition of the tumour suppressor PP2A and stabilizing the oncoprotein c-Myc. Treating MDA-MB-468 cells with FC results in the enhanced phosphorylation of CIP2a's mode III 14-3-3 binding motif at S904, the destabilization of c-Myc and the induction of apoptosis.

ABS-66449201

Synthesis of carnosine derivatives

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Carnosine is one of the most common dipeptides found in humans. It consists of β -alanine and L-histidine and possesses many functions, to mention antioxidant, chelating, anti-glycating, anti-aging and anti-neoplastic. Unfortunately, carnosine action is inhibited due to carnosinases – hydrolytic enzymes cleaving it quickly to amino acids, limiting therapeutic action of the dipeptide. To overcome this limitation, there's an urge to create derivatives possessing the same function as carnosine, but in the same time being immune to carnosinases [1,2].

Aims

The aim of the study was to synthesize properly protected carnosine derivatives in the form of methyl esters with an altered amino acid sequence relative to the native peptide.

Methods

We propose the synthesis of new derivatives of carnosine in the form of methyl esters. The synthesis scheduled oligopeptides Y- β -Ala-X-OMe and Y-X-His-OMe where e.g. X=His, Arg, Lys, Phe, Val, Lys; Y=Boc, Fmoc; conduct in a solution, accordance with the rules of peptide chemistry, by previously checked by our methods, eg. by the mixed anhydride with isobutyl chloroformate and N-methylmorpholine (NMM), or apply an appropriate reagent condensing, e.g. EEDQ, EDCI or T3P. Deprotecting from the amino group tert-butoxycarbonyl (Boc) of the protected peptide by trifluoroacetic acid (Tfa) and cover Fmoc by diethylamine. allow the nucleophilic substitution reaction of an aromatic ring acridine. Structures of the all obtained compounds will be confirmed ¹H NMR, ¹³C NMR, MS and elemental analysis techniques. Their purity will be established with HPLC.

Results / Conclusions

We obtained a series of oligopeptides in the form of methyl esters with the altered amino acid sequence of the native dipeptide. The modifications relate to the replacement of His or β -Ala with another amino acid. We introduced amino acid residues found in antioxidant peptides released from food proteins during digestion, fermentation and enzymatic processes that positively affect our health.

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ABS-66449203

Designing carnosine analogs based on molecular modeling

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Carnosine is a natural dipeptide with remarkably beneficial profile of biological activities, and therefore, it is clear that it should be used in designing of new drugs, including Alzheimer's disease treatment [1]. Although, so far have been synthesized series compounds from acridine group, including tacrine derivatives, still is being searched another analogues, which may bring us closer to receive more effective Alzheimer's disease medicines, with reduced hepatotoxicity, improved bioavailability, not rapidly eliminated from the cell and acting through the different cytophysiological mechanism. Computational methods are one of the stages of designing new compounds with biological activity.

Aims

Molecular modeling studies were carried out for a series of new carnosine analogues including: appropriate preparation of AChE / BChE enzyme structures available in the PDB database, preparation of chemical structures of ligands and optimization of their geometry, and molecular docking of optimized ligands to prepared receptor structures.

Methods

The selection of new inhibitors was performed based on the analysis of the determined free binding energy (using the AutoDock Vina 1.1.2 docking program). Visualization of the results of molecular docking allowed to identify potential sites for the interaction of new potential inhibitors with amino acid residues building the active sites of these enzymes [2].

Results / Conclusions

Conducted preliminary analyzes using molecular modeling techniques to show the theoretical potential of acridine analogs of carnosine as potential inhibitors AChE / BChE.

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Biologicals & Small Molecules

ABS-67517546

Modelling the pharmacokinetics of intravenous immunoglobulin in Guillain-Barré syndrome

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Introduction

Intravenous immunoglobulin (IVIg) is the treatment of choice for the Guillain-Barré syndrome (GBS). The working mechanism of IVIg in GBS is undefined, but most likely all potential effects are dose dependent. A rapid clearance of IVIg in the weeks following administration was previously shown to be associated with poor recovery. Yet it is unknown which factors contribute to the highly variable pharmacokinetics (PK) and pharmacodynamics (PD) of IVIg in GBS patients.

Objective

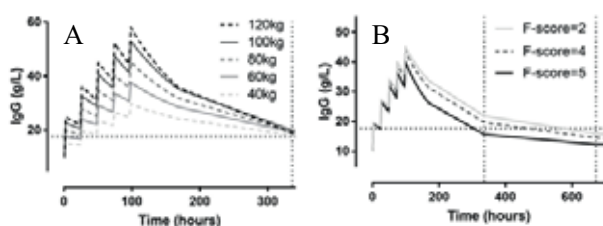
Identify factors that influence the PK of a standard dosage of IVIg (0.4 g/kg for 5 consecutive days) to individualize dosing for patients with GBS.

Methods

Non-linear mixed-effects modelling (NONMEM) was used to construct a model based on a cohort of 177 GBS patients, with a total of 811 sequential serum IgG levels. Model development was based on improvements in the objective function value, realistic parameter estimates, error estimates, shrinkage values and visual improvement of goodness-of-fit-plots as well as visual predictive checks. The effect of covariates on PK parameters was evaluated. Several dose regimens were evaluated by simulating a dataset of 1000 GBS patients.

Results

The final model containing 2 compartments and a baseline IgG level, accurately describes the day to day increment in IgG levels during the 5-day course and the initial rapid fall and gradual decline to steady-state levels thereafter. Bodyweight (fig A), GBS disability score (fig B) and methylprednisolone cotreatment were correlated with clearance.



Conclusion

As far as we are aware, the first accurate and robust NONMEM model for the PK/PD of standard IVIg treatment in GBS was developed. The model can be used to predict the PK in individual patients applying a few simple baseline characteristics. In addition, the effect of different treatment regimens of IVIg in GBS on a population PK/PD level can be simulated. This modelling technique is a new tool to optimize the PK in individual patients and study design for new trials with IVIg in GBS.

ABS-6617859

Structure Kinetics Relationships and Molecular Dynamics show crucial role for heterocycle leaving group in irreversible diacylglycerol lipase inhibitors.

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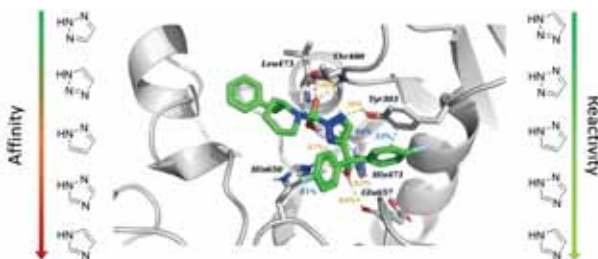
Drug discovery programs of covalent irreversible, mechanism-based enzyme inhibitors often focus on optimization of potency as determined by IC_{50} -values in biochemical assays. These assays do not allow the characterisation of the binding activity (K_i) and reactivity (k_{inact}) as individual kinetic parameters of the covalent inhibitors.

Aims

We aimed to study the kinetics of binding of irreversible Diacylglycerol Lipase inhibitors using biochemical methods.

Methods

We developed a kinetic substrate assay to study the influence of the acidity (pKa) of heterocyclic leaving group. As a test case, we synthesized set of triazole urea derivatives as diacylglycerol lipase (DAGL)- α inhibitors. The molecules were also analysed using molecular dynamics simulations while bound to a homology model of DAGL- α .



Results / Conclusions

We found that the reactivity of the inhibitors did not correlate with the pKa of the leaving group, whereas the position of the nitrogen atoms in the heterocyclic core determined to a large extent the binding activity of the inhibitor. This finding was confirmed and clarified by molecular dynamics simulations on the covalently bound Michaelis-Menten complex. A deeper understanding of the binding properties of covalent serine hydrolase inhibitors is expected to aid in the discovery and development of more selective covalent inhibitors.

ABS-66098613

Discovery of an in vivo active NAPE-PLD inhibitor that reduces brain anandamide levels and pain behavior

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Biologicals & Small Molecules

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N-acyl ethanolamines (NAEs) are a family of signaling lipids biosynthesized by the enzyme N acylphosphatidylethanolamine phospholipase D (NAPE-PLD). A lack of potent and in vivo active inhibitors for this enzyme has hampered the study of these signaling molecules. Here, we report **LEI-401** as the first brain-penetrant, in vivo active inhibitor for NAPE-PLD. **LEI-401** was identified through high-throughput screening and an extensive hit optimization campaign. **LEI-401** showed nanomolar potency ($K_i = 27$ nM) for NAPE-PLD in a biochemical substrate assay and was selective over the other proteins of the endocannabinoid system. In situ target engagement was established by chemical proteomics using an **LEI-401**-based two-step photoaffinity probe. **LEI-401** lowered a broad range of NAEs in a mouse Neuro-2a neuroblastoma cell line, but not in NAPE-PLD^{-/-} cells. Mice exhibited reduced NAE levels in the brain, including anandamide, two hours after i.p. administration of **LEI-401**. Furthermore, the compound induced locomotor depression, hypothermia and elevated hot plate latencies in the mouse tetrad assay, but no signs of catalepsy or anti-nociception in the tail flick assay. These effects were also observed in cannabinoid CB₁-receptor knock-out mice. In a mouse model for inflammatory pain, **LEI-401** was able to fully reverse lipopolysaccharide-induced allodynia. These findings suggest that lowering basal NAE levels has profound neurophysiological effects and induces an analgesic response via a non-CB₁-receptor mediated pathway.

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Synthesis and evaluation of triazolo-pyrimidinone derivatives as noncompetitive, intracellular antagonists for CCR2 and CCR5

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Introduction

Both CC Chemokine receptors 2 (CCR2) and 5 (CCR5) are involved in a variety of inflammatory and immunological

diseases; however, with the exception of maraviroc, clinical trials with selective CCR2 and CCR5 antagonists have been unsuccessful. Preclinical evidence suggests that dual CCR2/CCR5 inhibition might represent a more effective strategy for the treatment of multifactorial diseases. In this regard, the high conservation of a recently discovered intracellular binding site in chemokine receptors¹ provides a potential new avenue for the design of multitarget allosteric modulators.

Aims

In this study, we synthesized and evaluated the biological activity of a series of intracellular triazolo-pyrimidinone derivatives in both CCR2 and CCR5, with the aim to (i) develop structure affinity/activity relationships for both receptors, and (ii) gain understanding of the structural requirements to modulate selectivity.

Methods

Radioligand binding assays and a β -arrestin recruitment functional assay were used to determine the affinity and activity of the synthesized compounds on CCR2 and CCR5. The mechanism of inhibition of selected compounds was also investigated in both receptors using a [³⁵S]GTP γ S assay. Finally, an in silico approach was used to explore the binding mode of these compounds.

Results / Conclusions

Radioligand binding assays and the docking studies confirmed that triazolo-pyrimidinone derivatives bind to the intracellular site of CCR2 with high affinity. In general, the derivatives were mostly selective towards CCR2; however compounds **39** and **43** were able to inhibit CCL3-induced β -arrestin recruitment in CCR5 with ~100 nM potency. Overall, these findings indicate that even though the intracellular pockets of CCR2 and CCR5 are highly conserved, selectivity of intracellular ligands can be fine-tuned, allowing the design of either selective or multitarget ligands. Finally, these compounds displayed an insurmountable mechanism of inhibition in both receptors, which holds promise for improved efficacy in inflammatory diseases characterized by elevated levels of endogenous chemokines.

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ABS-66452551

Plants Bioreactors for Pharmaceuticals: A new approach for Expression of Tissue Plasminogen Activator (t-PA)

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Plasminogen activators are thrombolytic factors that digest fibrin to soluble products by converting the proenzyme, plasminogen, into plasmin that is an important enzyme present in blood and its deficiency may lead to thrombosis. tPA protein includes four domains (a serine protease domain a finger domain, Kringle 1 and Kringle 2 domains, a growth factor domain) [1]. Recently, plants have been introduced as cost-effective, safe and large-scale systems for production number of biopharmaceutical components like as anti-cancers [2-6]. The low amount of expression of recombinant protein in plants might be occurred, due to proteolytic activities in plant cells. This fact has been demonstrated in some studies including production of enzyme inhibitors and some antibodies in transgenic plants [7-12]. To overcome these problems, some solutions including organelle-specific targeting and tissue-specific expression have been suggested [6].



Aims

In this investigation, application of some targeting signals for delivery recombinant tPA to different cell compartments, were possible solution to decrease of protein lysis. Three signal peptides such as KDEL, Extensin and Sp (the first part of Zera signal) were used for protein delivery to ER, Apoplast and cytosolic spaces respectively [12]. This is the first report of the production recombinant tPA in different cell compartments of lettuce that they were active in terms of enzymatically activation.

Methods

Following construction of three final plant binary vectors (pBI-Ext-tPA, pBI-SP-tPA, pBI-KDEL-tPA cassettes), Leaf pieces of lettuce plant (*Nicotiana tabacum* cv. Xathi) were used for agrobacterium-mediated transformation by *Agrobacterium tumefaciens* strain LBA4404.

Presence of tPA gene was confirmed by PCR analysis of lettuce plants that were transformed by (pBI-tPA-KDEL), (pBI-SP-tPA), (pBI-Ext-tPA) constructs. For the Southern Blot analysis, about 40 µg of genomic DNA was digested with Hind III and separated by electrophoresis in a 0.9% agarose gel. Hybridization and detection were carried out using a DIG High Prime DNA Labelling and Detection Starter Kit (Roche) according to the manufacturer's instruction.

The amount of tPA protein in transgenic carrot root was determined by using an ELISA (enzyme linked immunosorbent assay). and an average expression level was obtained.

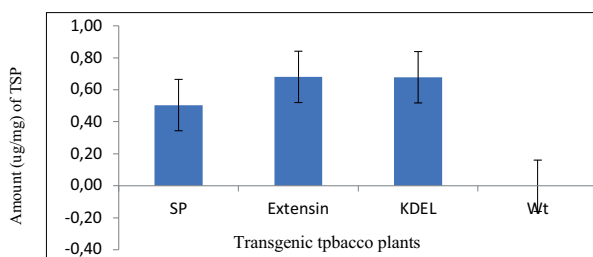
Enzymatically activity of rtPA was determined by Gelatine zymography.

Results

presence of the tPA gene were confirmed by PCR analysis. In brief, for lettuce plants that were transformed by pBI-SP-tPA construct amplified fragment was 2250 bp (tPA 1700bp- LTB 350bp- SP signal 120bp- CaMV35S promoter amplified 130bp), for lettuce plants that transformed by pBI -tPA-KDEL construct amplified fragment was 1830 bp (tPA 1700bp- CaMV35S promoter amplified 130bp), for lettuce plants that were transformed by pBI-Ext-tPA construct amplified fragment was 1950 bp (tPA 1700bp- Extensin signal 120bp- CaMV35S promoter amplified 130bp).

Southern blotting confirmed that some of the transgenic lettuce plants have more than one copy number of tPA gene that integrated into genomic DNA. Ten lettuce plants which by different constructs were transformed were investigated by southern blotting.

Using ELISA, immune-activity of recombinant tPA from lettuce plants were analysed. Normal immune-activity of rt-PA was observed. Amount of recombinant protein rtPA for lettuce plants were identified by ELISA analysis. The yield of recombinant tPA in lettuce for SP, KDEL, Extensin signals respectively 0.50, 0.68, 0.69 (µg/mg) of total soluble protein was obtained.



ELISA assay. Three hundred micrograms of total soluble protein were used for ELISA. Transgenic lettuce plants were transformed with different construct. Wilde type of lettuce was used for negative control.

Enzymatic activity of rtPA to convert of deactivate pro enzyme plasminogen to plasmin was determined using gelatine zymography and it was positive for separated proteins were produced in each of three cell compartments. This assay has been shown that enzymatic activity was conserved even after insertion various peptides to target of main protein to different compartments, although they had different activities. There was no activity in the control plants. In addition, Altplase was used as positive control.

Conclusions

To obtain high level of accumulation of proteins in plant cells, it is necessary to target heterologous proteins to different organelles to escape from hydrolytic activity of protease. Here, the reported active tPA proteins can be expressed in different cell compartments of transgenic lettuce plants using agrobacterium-mediated transformation. In some studies, targeting recombinant protein by signal peptides to the apoplast, mitochondrion, vacuoles, plastid and the nucleus have also been reported [13-14]. Apoplast (inter-cellular space beneath the cell wall), endoplasmic reticulum and cytoplasm can be targeted for expression recombinant proteins [12]. By targeting recombinant proteins to the apoplast or ER where they are protected from degradation by cytoplasmic proteases and the amount of recombinant proteins would further enhanced. For the ER lumen in plant cells and mammalian, KDEL is known as a retention signal [16]. The most post-translational modification of therapeutic occurs in ER and Golgi compartments. A greatly enhanced accumulation of the recombinant protein was being produced by presence of the ER-targeting signal. The recombinant protein is then destined for secretion to the apoplast, where it significantly accumulates to higher levels compared to the cytosol [12,15]. The range 0.5–2% TSP was expected for the standard expression levels of recombinant proteins for stably transformed plants in the apoplast and ER. In this study, the size of recombinant tPA was different for constructs, 85kDa for pBI-SP-tPA, 66kDa for pBI-tPA-KDEL, and 67kDa for pBI- Ext- tPA and 63 kDa for commercial tPA (Altplase) as positive control. Expressed-rtPA in different compartments showed enzymatically activity and was more active in apoplast (150 µg of TSP for lettuce had been used). In this report, we have applied this peptide in molecular farming. Results showed that the expression of the rt-PA in different compartments had enough quality and activity.

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ABS-66427363

Safety, Tolerability, Immunogenicity and Shedding of a Live-Attenuated Respiratory Syncytial Virus Vaccine in Healthy Adults

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Respiratory Syncytial Virus (RSV) is a leading cause of hospitalization of children under 5 years of age. Currently, there is no effective treatment for an ongoing RSV infection nor is there a safe and effective vaccine available. Therefore, Intravacc developed a live-attenuated recombinant RSV vaccine. With reverse genetics a virus was constructed lacking the G attachment protein (RSVΔG) resulting in a live-attenuated virus that induced a potent immune response in pre-clinical studies.

Aims

We performed a first-in-human, randomized, double-blind, placebo-controlled trial to assess the safety, tolerability, viral shedding and immunogenicity of RSVΔG in healthy adults.

Methods

Healthy adult volunteers (n=48) were randomized in a vaccine:placebo ratio of 3:1 to receive a single dose of 6.5±0.5 CCID50 RSVΔG or placebo intranasally. Subjects were eligible for inclusion if they had virus neutralization antibody titers (VNT) ≤ 9.6 log2. Safety was assessed by vital signs, routine blood chemistry and hematology assessment, nasal examination and oral temperature. Tolerability was assessed by a visual analogue pain scale. Symptom scores were derived from solicited adverse events (AEs) recorded by subjects daily on a mobile

application. Viral shedding was assessed by quantitative culture and PCR. Immunogenicity was determined both in serum (VNT, palivizumab competing antibodies) and nasal wash (VNT, IgA).

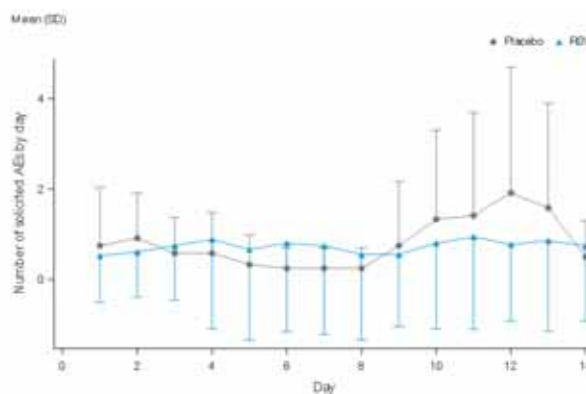


Figure: Mean (standard deviation [SD]) of number of solicited AEs per day for RSVΔG and placebo.

Results / Conclusions

Intranasal administration of RSVΔG was well tolerated and safety analysis did not show findings of clinical concern. All treatment emergent (TE) AEs recovered without sequelae during the first 28 days (mild [n=74], moderate [n=3], severe [n=0]). The number of solicited AEs (figure) and symptom scores were similar for the RSVΔG (n=36) and placebo group (n=12) (figure 1). No significant change in serum VNT was observed after vaccination compared to placebo. Analysis of other immunogenicity endpoints did not show an evident immune response. However, pre-existing immunity against RSV complicates the assessment of immunogenicity in healthy adults and can ultimately be best assessed in the RSV-naïve population. A dose of 6.5±0.5 log10 CCID50 could also be too low to elicit an immune response in healthy adults. Further research investigating the immunogenicity of RSVΔG (such as dose-escalation trials) appears to be warranted.

ABS-66030674

Ligand-target binding kinetics: A case for the ENT1 transporter

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Background

In the last decade it has been recapitulated that receptor-ligand binding kinetics, i.e. ligand association and dissociation to and from its target over time, is an additional relevant parameter in drug discovery.¹ For that reason, the traditional paradigm, mostly emphasizing on affinity and potency is being complemented with ligand-target residence time (RT), i.e. the duration of the ligand and target complex.²

Equilibrative Nucleoside Transporter-1 (ENT1), the most abundant nucleoside transport protein, mediates the facilitative diffusion of nucleosides along their concentration gradients. ENT1 inhibitors can potentially be used in the treatment of ischemic heart disease, stroke, viral infections and cancer.^{3,4} Therefore, this project is focused on the determination of affinity and binding kinetics of ENT1 inhibitors, in order to establish structure-affinity (SAR) and structure-kinetic (SKR) relationships.

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Experimental approach.

A radioligand displacement assay was developed for hENT1 using the radiolabeled inhibitor [^3H]NBTI to determine the affinity of ENT1 inhibitors. In addition, a [^3H]NBTI competition association assay was established to determine their binding kinetics. Finally, a label-free assay was used to evaluate the impact of divergent inhibitor binding kinetics in a functional assay.

Results

The affinity and binding kinetics of ENT1 inhibitors with four different chemical scaffolds were determined, yielding a wide variety in values for both parameters. Three of the scaffolds presented good affinities and short to moderate RTs (1-44 min). Contrarily, draflazine analogues (the 4th scaffold) showed longer RTs, with one analogue having a RT of over 10 h. Draflazine and the long RT analogue were functionally evaluated in a label-free assay. The long RT inhibitor presented a time-dependent inhibitory effect, which was not observed for draflazine, supporting the importance of binding kinetics in target-occupancy and duration of action.

Conclusions

Our research aims at finding new drugs targeting ENT1 transporter by incorporating kinetic binding parameters next to affinity. All in all, this approach could inspire future drug discovery in the field of membrane transporters.

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Identification and optimization of inhibitors of the calcium-dependent N-acyltransferase PLA2G4E

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In 2016, the enzyme PLA2G4E was discovered to be a calcium-dependent N-acyltransferase capable of producing N-acyl phosphatidylethanolamines (NAPEs) from phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE)¹. Together with the calcium-independent members of the phospholipase A/acyltransferase (PLA/AT) family, PLA2G4E is suggested to be the enzyme responsible for the production of NAPE in the endocannabinoid system (ECS).

Aims

The human endocannabinoid system is a complex network of receptors, their ligands and the enzymes involved in the biosynthesis thereof. Endocannabinoids are involved in regulating numerous processes including nociception, appetite, synaptic plasticity and immune responses. Gaining a deep understanding of the ECS could therefore prove to yield valuable information

to develop new ways of treating for example pain, obesity and neurodegenerative diseases². Little is known about the enzymes involved in the biosynthesis of the endocannabinoid anandamide, or its precursor NAPE. In this study, the first inhibitors of calcium-dependent NAPE production were discovered, providing tools to study the physiological importance of PLA2G4E.

Methods

In this study, two activity-based protein profiling (ABPP) assays were developed for PLA2G4E. Both a conventional gel-based assay and a fluorescence-polarization (FluoPol) assay were developed, making use of the broad-spectrum serine hydrolase probe fluorophosphonate-TAMRA. Gel-based ABPP allows for the visualization of multiple enzymes at the same time, whereas the FluoPol assay is performed in 96-wells format allowing for higher-throughput screening of inhibitor compounds.

Results / Conclusions

Using the two assays, an in-house compound library was screened against hPLA2G4E derived from HEK293T cell overexpression and inhibitors were identified. Based on these results, new molecules were designed, synthesized and tested for activity on PLA2G4E. Selectivity over PLA2G4 family members and other enzymes within the ECS was studied by gel-based ABPP. Via this approach, WEN091 was identified as a potent and selective inhibitor of PLA2G4E with an IC₅₀ of 10 nM. The activity and selectivity of this compound in living cells is currently being investigated.

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ABS-65703980

Development of MIF binders to protein-protein interaction inhibitors

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Macrophage migration inhibitory factor (MIF) is a versatile protein implicated in inflammation, autoimmune diseases and cancers.^{1,2} MIF mainly functions through binding to membrane receptors to activate downstream signaling pathways.³

Aims

To develop strong and selective binders for explore the role of MIF and also as anti-proliferation inhibitors.

Methods

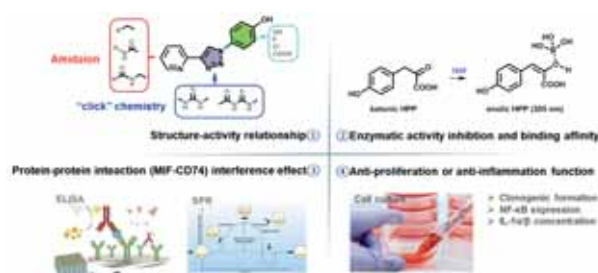


Figure 1. Methods used in this project. 4

Results / Conclusions

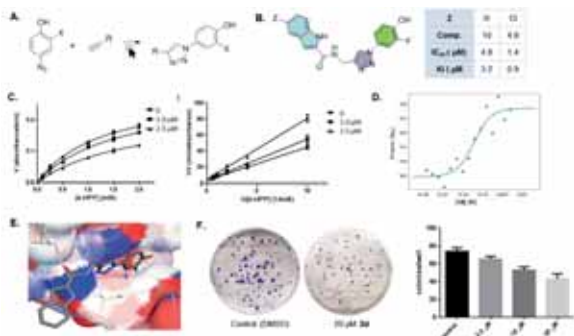


Figure 2. Synthesis and characteristics of MIF binders. A) MIF binders synthesized by "click reaction"; B) Inhibition potency against MIF tautomerase activity of 1d and 2d; C) Michaelis-menten and Lineweaver-Burk plots of 1d on MIF tautomerase activity; D) Affinity between 1d and MIF tested by MST; E) Binding between 1d and MIF rationalized by molecular modeling; F) Colony formation of A549 cell inhibited by 2d.

In this study, 23 phenyl triazoles derivatives were synthesized and their structure and activity relationships for MIF binders that interfere with the MIF tautomerase activity was investigated. The most potent two inhibitors in this series **1d** and **2d** show K_i values of 3.2 and 0.9 μM respectively. Enzyme kinetic analysis indicate competitive inhibition and the binding model can be rationalized by molecular modelling. Binding of **1d** was confirmed MST with K_d value of 3.2 μM . The growth inhibitory effect of **2d** was measured by colony formation assay and it exhibited anti-cell growth effect with a dose-dependent manner. Altogether, this study provides insight in the structure-activity relationships for phenol triazole and further validates the potential of this class of compounds as MIF-receptor interaction inhibitors.

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ABS-67592179

Pharmacological challenge models in clinical drug developmental programs

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Early phase clinical research for drug development requires the investigation of safety, tolerability and efficacy. The latter is hampered by the absence of the disorder in healthy volunteers which is why challenge models are often applied in order to demonstrate 'proof of pharmacology' of novel compounds. These challenge models can often be translated from animal work and can inform the drug developer which dose, dosing regimen or application frequency should be selected prior to phase 2 studies in the target population. Additionally, these challenge models represent well-controlled settings to perform activity screening of the compound.

Aims

To evaluate current literature on local skin challenge models and to provide an oversight on caused immune responses and pharmacodynamic effects of these models.

Methods

A non-comprehensive literature search resulted in approximately 100 articles deliberating ten skin challenge models in the categories: inflammation, itch and UV-exposure. The following four models were selected for evaluation: imiquimod induced skin inflammation, histamine and cowhage provocation, UV-B skin irradiation and KLH induced skin inflammation.

Results / Conclusion

All four models caused the desirable expected effect, i.e. inflammation, itch, skin irradiation and hypersensitivity, respectively. However, only the imiquimod and KLH skin challenge model were well described and assessed with a toolbox of imaging, biophysical, clinical, cellular and molecular measurements. Topical application of imiquimod resulted in a quick innate immune response followed by an adaptive response while histamine and UV-B irradiation only generated innate immune responses. KLH induced only an adaptive immune response (Table 1). In conclusion, all four evaluated models seem appropriate for future proof-of-pharmacology of novel compounds. Depending on the expected mode of action of the novel compound, the right challenge model can be chosen.

Table 1. Overview of human skin challenge models

CHALLENGE	APPLICATION	MODE OF ACTION	CONDITION INDUCED	IMMUNE RESPONSE
INFLAMMATION				
IMIQUMOD	Local under occlusion	TLR7 agonist	Local inflammation	Innate followed by adaptive
KLH	Intradermal, Intramuscular	Neo-antigen	Local inflammation, systemic immune response	Adaptive
ITCH				
HISTAMINE	Intradermal, intramuscular	H1,2,3,4 receptor C ₁ fibers	Itch	Innate
COWHAGE	Cutaneous	CMH-fibers	Itch Burning	Unknown
UV-EXPOSURE				
UV-B IRRADIATION	LOCAL Thermode	PI3K/AKT/mTOR-upregulation	Pain, pigmentation, erythema, inflammation	Innate/Adaptive

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Development 1 - Clinical Trials

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ABS-65236770

A randomized, double-blind, placebo-controlled, single dose, 3-way cross-over study in healthy elderly subjects to develop an anti-cholinergic pharmacological challenge with biperiden

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Introduction

Currently, several selective M1 muscarinic acetylcholine receptor (M1 mAChR) agonists are under development as symptomatic treatment for the cognitive effects of Alzheimer's disease. In early phase development of these drugs, usually performed in healthy subjects, it is difficult to demonstrate acute pharmacodynamic (PD) effects, eg on cognitive function, due to ceiling effects of the PD tests in healthy subjects. A challenge model affecting the mAChR that induces temporary reversible cognitive deficits could be used for proof-of-pharmacology of new M1 mAChR agonists. Biperiden is a selective M1 antagonist that passes the BBB and therefore a suitable challenge. Investigating a biperiden challenge model with repeated PD and PK measurements in elderly subjects was not done before(1).

Objectives

- To determine the profile of effects on the central nervous system at several time points after 2 mg and 4 mg biperiden in comparison to placebo.
- To determine the pharmacokinetics (PK) of biperiden
- To determine the plasma concentration-effect relationship of biperiden on NeuroCart tests using PKPD modeling.

Methods

In this 3-way cross-over study, biperiden (2 mg and 4 mg) and placebo were administered to 12 healthy elderly subjects (65-80 years, MMSE \geq 28). Pre-dose and post-dose PD was repeatedly assessed using the NeuroCart battery of tests (adaptive tracking test, n-back, eye movements, visual verbal learning test (VVL), tapping, VAS Bond and Lader, VAS nausea, body sway, pharmacological EEG, mismatch negativity)(2). PK/PD analysis is ongoing.

Results / Conclusions

Results show that biperiden had consistent dose dependent effects on tests of learning and memory. Differences from placebo were mainly significant after administration of 4 mg biperiden. The reaction time on the n-back test increased up to 49.9 msec (95% CI [21.854; 77.882], $p=0.0016$) and the adaptive tracking (sustained attention) performance was consistently impaired (mean difference up to -2.095%, 95% CI [-3.043;-1.148], $p<0.001$) compared with placebo. In the VVL (memory), significantly fewer words were recognised (mean difference -6.5, 95% CI [-10.8; -2.2], $p=0.0053$) or recalled (mean difference -3.1, 95% CI [-5.9; -0.2], $p=0.0344$) compared with placebo. These results support the idea that this biperiden challenge model

can be used for proof-of-pharmacology and that it is feasible in elderly subjects.

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ABS-67596613

Pharmacological characterization of the novel selective orexin-1 receptor antagonist JNJ-64393215 in healthy subjects

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Introduction

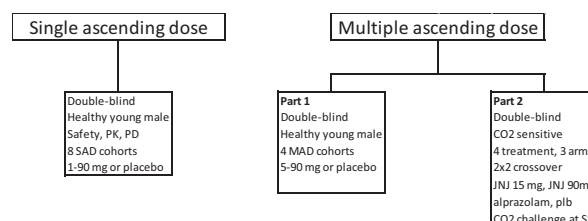
The neuropeptide orexin A is an endogenous ligand for the orexin-1 receptor (OX1R), which occurs in central nervous system regions regulating fear, anxiety and reward. Preclinical experiments with the new, selective OX1R antagonist JNJ-6139215 (JNJ) demonstrated anxiolytic-like effects in several fear and anxiety-related behavioral paradigms. Also, JNJ blocked CO₂-induced anxiety behavior in the social interaction test in rats. These findings support a potential role of OX1R inhibitors as a treatment strategy for fear and anxiety-related neuropsychiatric disorders.

Objectives

The current study investigated the safety and tolerability of JNJ, and its clinical anxiolytic effects compared to the anxiolytic drug alprazolam using a validated panic-inducing CO₂ inhalation challenge in healthy male subjects.

Methods

A schematic representation of the design and different parts of the study are depicted below.



In the MAD part 2, CO₂ sensitive subjects were randomized to either JNJ 25mg, JNJ 90mg, alprazolam 1mg bid or placebo for 7 days in a partial cross-over fashion. On day 7, a double-inhalation 35% CO₂ challenge was administered.

Results

66 (mean age 26.2) and 71 healthy men (mean age 29.2) were included in the SAD and MAD studies, respectively. 39 subjects

Development 1 - Clinical Trials

underwent the CO₂ inhalation challenge. Following single doses ranging from 1 to 90 mg JNJ-6139, C_{max} increased from 97.4 to 4497ng/mL and was dose-proportional up to 30mg. Median t_{max} was 1.0- 1.5h and 2.3h at doses below and above 30mg, respectively. Terminal half-life ranged from 13.6- 24.6h. After 7 days of dosing, accumulation for C_{max} and AUC_{24h} ranged from 95%-145%, and 77%-160%, respectively. Under non-challenged conditions, JNJ did not affect alertness in both SAD and MAD. Following the CO₂ challenge, JNJ significantly reduced panic symptoms using the Panic Symptoms List-IV (PSL-IV), which was comparable to alprazolam (LS Means difference -2.3; p<0.02 and -3.4; p<0.03 respectively). JNJ was well tolerated. All TEAEs were mild and self-limiting. Most common were somnolence and headache, with no clinically relevant effects on safety blood chemistry or hematology, ECG or vital signs.

Conclusions

These results support the safety and tolerability of the selective OX1R antagonist JNJ-61393215 in humans up to 90mg multiple dose administration. Moreover, it reduced CO₂-induced panic at 90mg following multiple dosing, which was comparable with alprazolam at a therapeutic dose. Together, these data support testing the efficacy of JNJ in patients with fear and anxiety.

ABS-66451925

Evaluation of the human intradermal LPS challenge model with topical and systemic corticosteroids

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Background

Intravenous lipopolysaccharide (LPS) administration to humans is a widely used, safe and well-tolerated model to study TLR4-driven inflammation. A local LPS challenge, in the skin, would offer the advantage of repeated testing within one subject, in a relevant peripheral tissue. We evaluated whether the inflammatory response following an intradermal LPS injection, as characterized before by various imaging, cellular, and biochemical techniques, can be suppressed using either topical or systemic corticosteroids.

Methods

In total 30 healthy volunteers participated in this trial. 24 subjects received a two day pretreatment on a designated area on the volar forearm with clobetasol propionate 0.05% topical formulation BID. 6 subjects received a two day pretreatment with oral prednisolone at 0.25mg/kg BID. Subjects received maximally 4 LPS injections (10ng LPS in 100µL saline/injection) on designated areas on the volar forearm. Non-invasive measurements included laser speckle contrast imaging (LSCI), thermography, and clinical erythema grading scale. Suction blisters were induced at different time points to analyze inflammatory cells and cytokines in blister fluid. Skin not exposed to LPS served as control.

Results

Both clobetasol propionate and prednisolone pretreatment caused a significant reduction in LPS-driven enhancement of skin perfusion as measured with LSCI, this effect was maximum (-50%) at 24h (38.8 AU ± 13.7 for control, 20.9 AU ± 12.7 for prednisolone, and 17.2 ± 9.7 for clobetasol propionate). In contrast, the corticosteroids did not impair the acute cellular and cytokine response to LPS. However, at 24 and 48 hours after LPS injection, clobetasol and prednisolone significantly reduced cell numbers in blister fluid compared to the untreated areas, as

observed for monocytes, dendritic cells, and T lymphocytes, but not for neutrophils.

Conclusions

We demonstrated that both local and systemic corticosteroids modulate LPS-driven skin responses in healthy volunteers. These data support the LPS skin challenge as clinical model for the assessment of novel anti-inflammatory compounds.

ABS-66197893

The relation between factor VIII activity and factor VIII plasma concentration in patients with hemophilia A

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Introduction

Hemophilia A is a hereditary factor VIII (FVIII) deficiency, bleeding disorder. The biomarker FVIII activity is currently used to assess disease severity and to monitor treatment. FVIII activity is mainly measured by the one-stage clotting assay (OSA) which is simple and rapid, but has problems in sensitivity and specificity that can result in misclassification of disease severity and suboptimal treatment. Measurement of the FVIII concentration in plasma with LC-MS/MS might overcome these challenges. However, it is unknown how these two methods relate.

Objective

is to investigate the relation between FVIII activity and FVIII plasma concentration in patients with hemophilia A, as well as determinants for differences.

Methods

The cross-sectional study was conducted at the UMC Utrecht. All patients with hemophilia A receiving standard-of-care treatment were eligible for inclusion. 15-20 samples within each of the clinically used FVIII activity categories (<1%, 1-5%, 5-40%, 40-150%, 150-600%) were randomly selected and FVIII concentration (LC-MS/MS) was measured and compared to FVIII activity (OSA) with linear regression, Bland Altman (BA) analysis and a mountain plot. Potential determinants for differences between the two methods were obtained from medical records and analyzed with linear regression.

Results

87 samples were included. With BA analysis a mean difference of -1.3% between the two methods was found, the mountain plot was centered around zero. In samples with anti-FVIII antibodies the mean difference (between the methods) significantly increased with 133% 95% CI (79; 187) and in samples with turoctocog alfa -39% (-71;-8) or efmoroctocog alfa -49% (-97;-1) when compared to samples without these determinants.

Conclusions

Overall a good relation between the two methods was found. However anti-FVIII antibodies or B-domain modified products might result in differences with potential clinical impact. More research is needed to determine the value of FVIII concentration in addition to FVIII activity.



Development 1 - Clinical Trials

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ABS-67724226

Towards quantitative systems pharmacology models for coxibs targeting colorectal cancer

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Background

The COX-2-dependent Prostaglandin (PG) E₂ production plays a predominant role in colorectal cancer (CRC). PGE₂ modulates a number of signal transduction pathways that may affect proliferation, apoptosis, angiogenesis, immune responses, cellular adhesion, differentiation, and tumor invasion. COX-2 gene expression is up regulated in most human CRC. Cyclooxygenase (COX) inhibitors (COXIBs) have been proven to be promising agents for chemoprevention of CRC. The suppressive effect of these agents on tumorigenesis has been supported by a number of epidemiological studies, clinical trials, and animal studies.

Aims

To develop Quantitative Systems Pharmacology models (QSP) to predict the intestinal/colonic drug distribution and efficacy of COXIBs targeting CRC. To that end, we first investigated the intracellular pathways that can be modified by COXIB action. The next step will be to build a physiology-based (PB) pharmacokinetic (PK) model with a developed advanced compartmental gut absorption module able to predict the concentration of drugs in the gut tissue and in the enterocytes (intracellular). Finally we aim to bridge the PKPB model predictions and the effects on targets nodes of the pharmacological pathways.

Methods

A thorough literature search was made on potential modes of intracellular actions of three COXIBs, being Celecoxib, Sulindac and Aspirin and a network of potential targeted pathways has been drawn. Next, a Boolean modelling analysis and simulations have been performed based on the findings of the pathways investigations. We have used R Software and the SPIDDOR packages to perform the Boolean simulations of the states of this network with, as final outputs, levels of activation of cell proliferation and apoptosis. To this end, a standardized and automatized method to write the Boolean function has been created, consisting in a specific database structure and a translator R script. We were able to simulate several scenarios of which healthy subjects, Familial Adenomatous polyposis (FAP) Patients, and treatment by COXIBs. For building the PBPK model we use PKSim®/Mobi® software from Open System Pharmacology.

Results

It was found that in total 6 pathways can be modified by these drugs. Apart of COX dependent mechanism of action of COXIBs (direct diminution of PGE₂ production and diminution of EP₂ receptor activation, a G protein-coupled receptor) there were also COX independent pathways targeted by this class of drugs (WNT pathway, AKT pathway, PDE5/PKG/JNK1 kinases pathway, caspase apoptosis pathway, NF- κ B pathway). As the dysregulation and accumulation of β -catenin in the cytosol is one of the triggering event of the carcinogenesis for colorectal cancer, the WNT pathway seems to be the central key component of the system pharmacology network since its role is the down-regulation of the β -catenin intracytosolic level. Most of the others pathways mentioned, including the PGE₂/EP₂ signalling pathway, are all linked to the WNT pathway and seem to have an impact on the proliferation β -catenin mediated and apoptosis. The simulations performed based on this network of pathways allowed us to mimic the increase of proliferation observed in FAP patients and the reduction of carcinogenesis in case of treatment by COXIBs. The first PBPK simulations for Celecoxib in mouse were able to fit preliminary observed concentrations in plasma, but generated important discrepancies between predictions and observed concentrations in gut tissue and enterocytes.

Conclusion

Due to cross-links between the 6 pathways, we consider the whole system as a network and each drug can be active on several nodes. This should form the basis of the to be developed QSP models to predict the effect of COXIBs on Colorectal carcinogenesis at the molecular level. Preliminary data in mouse in the gut suggests an accumulation of Celecoxib in the colon. The PK profile in the plasma and colon are not paralleled suggesting that the plasma PK is not the only driver of the colon PK. This indicates that the actual PBPK model must be refined.

ABS-67614677

Drug absorption in early childhood: elucidating the role of the small intestine

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Introduction

The absorption of drugs is different between children and adults, due to numerous factors. Systemic drug exposure is for example augmented or restrained by drug transporters located in the epithelial lining of the intestine. Adverse events in pediatric drug therapy point to a change in the activity of these transporters from birth to adolescence. As the majority of drugs are given to children orally, efficient and safe pediatric pharmacotherapy is hindered by our finite knowledge about the activity of transporters in this specific population.

Objectives

The aim of this project is to compare the activity of transporters involved in the intestinal absorption of drugs in pediatric patients and adults.

Development 1 - Clinical Trials

Methods

The ex vivo Ussing chamber technique with pediatric ileum from atresia patients is applied to evaluate intestinal drug absorption, while corresponding adult tissue is used as control. The major intestinal transport routes (paracellular, transcellular) and efflux transport proteins (P-gp, BCRP) are probed by a combination of drug molecules. Apparent permeability (*P_{app}*) values are determined by periodic sampling of the donor and receiver compartments and drug concentration analysis by LC-MS/MS. Efflux ratios (ERs) are calculated from *P_{app}* values. Viability, functionality and integrity of the tissues are monitored with the aid of electrophysiological parameters (potential difference, short circuit current and transepithelial resistance).

Results

The Ussing chamber method has been successfully applied for both adult and pediatric ileal tissue. Samples from six children (age range: 8 weeks to 17 years) and three adults have been analysed. The ERs were 1,28 and 0,84 for the paracellular substance, while 1,20 and 0,85 for the transcellular marker compound in pediatric and adult tissues, respectively. The substrate for P-gp showed an ER of 2,02 and 1,76 while the value was 1,63 and 1,44 for the BCRP transported drug in pediatric and adult tissues, respectively.

Conclusion

The preliminary results show that for the tested patients paracellular and transcellular transport in pediatric and adult tissue is rather comparable. Moreover, P-gp and BCRP efflux was observed, but with the current data set no significant difference could be detected. The Ussing technique presents a solid and highly feasible approach to study intestinal drug transport in children. The method is suitable for the evaluation of the effect of active efflux transport in intestinal drug disposition.

ABS-67542729

Double-blind Response Test with phenytoin 10% and placebo cream for the identification of responders for the treatment of peripheral neuropathic pain as a personalized medicine approach

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Introduction

Topical analgesics are increasingly popular to treat neuropathic pain, especially due to good tolerability without systemic side effects. The fast pain reduction (within 30 minutes) after application of phenytoin cream as a topical analgesic for the treatment of peripheral neuropathic pain (PNP) provoked us to develop a fast response test. PNP is mostly symmetrical, mainly felt in the feet, and most often with same pain intensity in both feet. A single-blind response test (SIBRET) was developed. Responders were defined as patients who experienced within 30 minutes at least 2-points difference as scored on the 11-point numerical rating scale (NRS) between the phenytoin cream and the placebo cream applied areas, in favor of the former. To further objectify the test, we choose to proceed developing a double-blind response test (DOBRET). This test is seen as the best method to identify responders and non-responders. We felt this test can contribute to individualizing therapy in a most ethical way.²

Aims

To evaluate DOBRET in patients with PNP.

Methods

Between September and November 2018, 14 patients with peripheral neuropathic pain were tested with DOBRET using phenytoin 10% and placebo cream. The label of phenytoin and placebo cream tubes were covered with a sticker and codes (e.g. 1A, 1B). These patients suffered from different causes of PNP (see table 1). DOBRET was only performed when a patient had maximally 1 point difference on the NRS between 2 painful areas. Before and 30 minutes after cream application patients were asked to score their pain in both areas on the NRS. After this, unblinding followed and the stickers of the tubes were removed. Responders were defined as patients who experienced within 30 min at least 2-points pain reduction from baseline on the NRS in the phenytoin 10% cream applied area, and at least 1-point difference on the NRS between active cream and placebo cream area.

Descriptive statistics were used. To compare means of pain reduction after the application of phenytoin 10% and placebo cream on different areas in the same patient, the Wilcoxon signed-rank test was performed (matched pairs). We calculated the number of patients achieving minimum pain relief (MPR) from baseline of 30% (moderate benefit: MPR30) and 50% (considerable benefit: MPR50) measured on the NRS. The statistical analysis was performed using SPSS 22 (SPSS Inc., Chicago, IL, USA).

Results

The tested population consisted of 50% female. The mean age was 60.1 years (SD 16.6). The mean duration of pain was 5.4 years (SD 6.7). In table 1 diagnoses are presented. Comparing the mean pain intensity reduction of the whole group between phenytoin 10% and placebo applied areas, the pain reduction was 1.8 (SD 1.7) and 1.3 (SD 1.7), respectively ($p=0.14$). Other characteristics can be found in table 2. When stratifying on the defined definition of a responder 6 out of 14 patients (42.9%) were responders. In the responder group the mean pain intensity reduction at the phenytoin 10% and placebo applied areas were 3.1 (SD 0.7) and 1.8 (SD 0.9) respectively ($p<0.03$). One patient was a true placebo responder and felt 50% pain reduction in the placebo cream applied area, whereas in the phenytoin 10% cream applied area no change in pain was present. However, the in this patient diagnosed as SFN no biopsy was taken, and he also suffered for sciatic pain due to a bulging disk, which might have interfered with his response. Without this outlier, the mean percentage pain reduction of the phenytoin 10% and placebo cream applied areas are 34.6% (SD 30.9) and 18.9% (SD 31.9) respectively with $p = 0.01$. Calculation with the outlier does not reach statistical significance. Another patient experienced complete pain reduction in both feet (from 5 to 0 on the NRS).

Table 1 Diagnosis

Diagnosis	N
Chemotherapy induced polyneuropathy	4
Idiopathic peripheral neuropathy	4
Painful Diabetic neuropathy	3
Chronic Idiopathic Axonal Polyneuropathy	2
Small Fiber Neuropathy	1

Table 2 Comparisons of effect between phenytoin 10% and placebo cream application.

Characteristics and Effect	Phenytoin 10%	Placebo
Pre-treatment NRS (SD)	6.0 (1.3)	5.9 (1.3)
Post-treatment NRS (SD)	4.2 (2.2)	4.7 (2.0)
Mean pain reduction % (SD)	32.1 (31.1)	21.1 (31.8)
MPR50 % (N)	35.7 (5)	21.4 (3)
MPR30 % (N)	50.0 (7)	35.7 (5)



Development 1 - Clinical Trials

Discussion

This is the first report on DOBRET with phenytoin 10% and placebo cream. When comparing with SIBRET,¹ less pronounced effect occurred. This most probably is due to blinding the treating physician. The ethical issue whether or not using placebo is discussed elsewhere.² In short, when helping the patient to find the right treatment, placebo is justified using in a test in practice.

Conclusions

DOBRET is a new aspect of personalized medicine. The treatment is used as a test method with placebo to define responders. Future research will elucidate the optimal cut-off value of the DOBRET to predict long-term pain relief.

Conflicts of Interest

The authors are holders of two patents: (1) topical phenytoin for use in the treatment of peripheral neuropathic pain and (2) topical pharmaceutical composition containing phenytoin and a (co-) analgesic for the treatment of chronic pain.

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ABS-66450904

Finding suitable clinical endpoints for a potential treatment of a rare genetic disease. The case of ARID1B

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The treatment of patients with intellectual disability (ID) is challenging and usually symptomatic. In general, curative or causational treatments are not available. Pre-clinical studies have identified potential treatments for children with specific syndromes, e.g. clonazepam for ARID1B-related intellectual disability. ID trials have generally relied on subjective and variable endpoints, such as IQ measurement and questionnaires, which are unsuitable for early drug research.

Aims

The aim of this study was to assess the suitability of non-invasive clinical endpoints for ARID1B-related intellectual disability. Criteria for an ideal biomarker were: (1) Known to be sensitive to central nervous system effects of the pharmacological intervention, (2) feasible to perform multiple times in this population, (3) stable over time in the absence of an intervention and (4) A significant difference between control subjects and patients. Ideally, endpoints should correlate with known parameters of disease.

Methods

We performed a study with 12 subjects with ARID1B and 12 healthy age-matched control subjects aged between 2 and 31 years old. All subjects performed a battery of 6-13 tests, depending on age group. Tests investigating visuomotor skills and memory were included, as well as several EEG assessments. All tests were performed on two separate study days and were repeated on the same day to determine intra- and inter-subject coefficients

of variability. A mixed model analysis was performed to assess differences between ARID1B and healthy subjects. Mean test outcomes were correlated with the Aberrant Behavior Checklist (ABC) questionnaire and IQ.

Results / Conclusions

We were able to adequately conduct 11 out of 13 tests in this population. Variability was high for EEG tests but acceptable for visuomotor and memory tests. There was a significant difference between ARID1B subjects and controls for adaptive tracking, finger tapping, smooth pursuit eye movements, body sway and animal fluency test, as well as visual evoked potentials. We have identified these tests as candidate pharmacodynamic endpoints for early stage drug trials in ARID1B-related intellectual disability. The candidate endpoints will be evaluated in a study investigating the effects of clonazepam in this population.

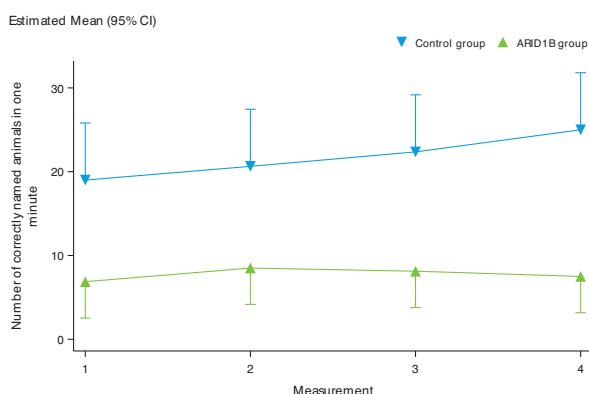


Figure 1. Least square mean plot of the animal fluency test for ARID1B and control subjects.

ABS-66419418

Peripheral neuropathy and hypovitaminosis D are associated in multiple myeloma patients

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Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a common adverse event in patients with multiple myeloma (MM), which decreases quality of life and requires dose adjustment, delay or premature termination of treatment, resulting in a negative influence on time to progression and survival. In addition, several studies have found that up to 54% of MM patients have peripheral neuropathy (PN) at diagnosis. A possible mechanism of vitamin D deficiency and PN was found in animal trials, where an increase of nerve growth factor was observed in diabetic rats after supplementation of vitamin D. Furthermore, correction of hypovitaminosis D through vitamin D supplementation was

Development 1 - Clinical Trials

found to reduce PN in patients with DM type 2.

Objectives

The aim of this study was to determine the association between vitamin D and the occurrence of PN in MM patients.

Methods

Smoldering and symptomatic MM patients in the Medical Centre Leeuwarden and Deventer Hospital, older than 18 years and able to give informed consent, were included in the study, regardless of stage or previous treatment. Blood samples were collected to determine vitamin D levels, and hypovitaminosis D was defined as a 25-hydroxyvitamin D level (vitamin D) below 75 nmol/L. The Indication for Common Toxicity Criteria (CTC) Grading Peripheral Neuropathy Questionnaire (ICPNQ), a validated questionnaire to distinguish different PN grades in MM patients, was used to determine the severity of PN. Visual Analog Scale (VAS) scores were used to grade the intensity of PN.

Results

We included 120 MM patients with a median age of 68 years (range 48 to 84), and 57.5% were male. The median vitamin D level was 49.5 nmol/L (range 10 to 138), and 84% had a serum 25-hydroxyvitamin D level <75 nmol/L. The percentage of patients with PN grade 1 or higher was 69%. In the medical records, absence or presence of PN was mentioned in 40% of the patients by clinicians. A trend was found between lower vitamin D levels (grouped <25, 25-49.9, 50-74.9, and ≥75 nmol/L) and higher incidence of PN ($p = 0.036$).

Conclusions

PN and hypovitaminosis D are common in MM patients, and low vitamin D levels are possibly associated with the occurrence of PN. In addition, more attention for PN is needed, as PN is underreported by clinicians. Further research is necessary to investigate the relationship between vitamin D and PN.

ABS-67583611

RCT results: Topical digoxin and furosemide gel for patients with external anogenital warts

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Introduction

Anogenital warts (AGW) are caused by low-risk human papillomavirus (HPV) types and represent the most common sexually transmitted viral disease. Current therapies for AGW have notable side effects and high recurrence rates. DNA viruses such as HPV rely on cellular K⁺ influx. Ionic contra-viral therapy (ICVT) comprised of digoxin and furosemide inhibits the K⁺ influx and is therefore a potential new treatment for AGW.

Objectives

A randomized, controlled trial was performed to assess safety and tolerability and explore pharmacodynamics and clinical efficacy of ICVT in patients with AGW.

Methods

Twenty-four patients with at least 3 external AGW were randomized to either ICVT or placebo (ratio 3:1) and administered

the gel once daily for 42 consecutive days. To assess safety and tolerability, laboratory safety testing was performed and adverse events, vital signs and ECGs were monitored. Clinical efficacy was assessed by lesion count and dimensions, measurement of viral load, HPV expression and histology. Patient-reported outcomes and quality of life (QoL) were assessed with use of an e-diary and paper questionnaires.

Results

ICVT was well tolerated as there were no clinically relevant safety findings and no serious adverse events. All adverse events (N=17) were of mild severity and self-limiting. No between-group differences in lesion count, dimensions, viral load, patient-reported outcomes and QoL were observed after treatment.

Conclusion

ICVT is safe to be administered in patients with AGW but shows no pharmacodynamic activity or clinical efficacy after 6 weeks of treatment.

ABS-66447523

Maturation of blood-brain barrier drug efflux transporters in the pediatric brain

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Introduction

When drugs exert their effects in the brain, extrapolation of doses from adults could be harmful for children as the blood-brain barrier (BBB) and blood-CSF barrier (BCSFB) function is still immature. For example, P-glycoprotein (Pgp/MDR1)-mediated transport of drugs out of the BBB appears age-dependent. As human data is scarce, we studied developmental variation in human BBB and BCSFB transporters.

Methods

Age-dependent variation in localization and staining intensity of the ABC transporters Pgp, breast cancer resistance protein (BCRP) and multidrug resistance proteins 1, 2, 4 and 5 (MRP1/2/4/5) was investigated using immunohistochemistry in post mortem brain tissue derived from 52 fetuses, neonates and children between gestational age 13-42 weeks and 0-3 years of postnatal age and adults. Staining intensity of transporters in cortex microvessels (BBB) and choroid plexus (BCSFB) was analyzed by semi-quantitative scoring.

Results

Immunostaining was detectable for Pgp, BCRP, MRP1, and MRP2 in microvessels. Staining intensity was higher for Pgp and BCRP in adult brain compared to fetuses, neonates and children. In contrast, MRP1 and MRP2 staining intensity was higher in fetuses, neonates and children. Choroid plexus was positively stained for Pgp, MRP1, and MRP2 and did not show age-related differences. MRP4 and MRP5 were not detected in brain cortex microvessels and choroid plexus.

Conclusion

BCRP, Pgp, MRP1, and MRP2 were detected in microvessels and choroid plexus of human fetal and pediatric brain and staining patterns appeared to be dependent on location and age.



Development 2 - Method Development

ABS-66441084

Combined efforts of in vitro-derived data and computational chemistry for drug-induced liver injury prediction

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Drug-induced liver injury (DILI) is one of the main reasons of drug attrition during clinical trials and of drug withdrawal from the market¹. This makes the early identification of hepatotoxicity of compounds a critical challenge. In silico hepatotoxicity prediction models make a cost-effective approach able to prioritize compounds for preclinical and clinical studies. Recent efforts have been made to create more accurate quantitative structure-property relationships (QSPR) models relating hepatotoxicity to chemical structure features². However, to date only few have integrated in vitro quantitative concentration response mechanistic data to their models³; high content imaging allows for such detailed quantitative mode-of-action information.

Aims

This study aims at integrating in vitro activation signal of stress response pathways involved in DILI to standard molecular description of compounds to better identify sub-structures that contribute to the liability for DILI

Methods

A library of 103 drugs with different liability for DILI was screened on previously established HepG2 BiP/Chop, p21/BTG2, HMOX1/SRXN1, ICAM1 and HSPA1B BAC-GFP reporter cell lines⁴. These cell lines are respectively associated with endoplasmic reticulum (ER), DNA damage, oxidative, inflammatory and heat-shock stress response pathways involved in DILI. Confocal microscopy images were obtained at 24, 48 and 72 hours after addition of the compounds (concentration ranging from 1 to 100 cmax). Additionally, propidium iodide (PI) and annexin V (AnxV) staining was performed to detect necrotic and apoptotic cells. Quantitative multiparameter image analysis was performed with CellProfiler. GFP integrated signal above 1, 2, 3 and 4 times the median, as well as cmax, AnxV and PI values were used as descriptors of the compounds along with physicochemical and topological descriptors of the compound structures. Gradient Boosted Decision Tree classification models were built from 70% of compounds to relate both in vitro derived data and molecular structure description to the FDA DILI annotation converted to binary scale (Most and No-DILI-concern). The binary set was balanced using oversampling. Finally, models were tested on the internal set consisting of the remaining 30% of compounds.

Results / Conclusions

So far, the results show that stacked modelling built from in vitro-derived data combined with physicochemical and topological descriptors of the compound structures created highly predictive models. Sensitivity 0.96, Matthew's correlation coefficient 0.88 and specificity 0.95.

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ABS-66453349

Photo biomodulation for wound healing

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Introduction

Wounds that are difficult to heal represent a serious health problem. The lesions severely affect the quality of life of individuals; they can also cause emotional damage and poses huge burden on healthcare. In the Netherlands, despite the use of modern wound treatment techniques, approximately 500,000 patients are suffering from complex and slow healing wounds. Slow healing wounds are a growing challenge that requires innovative strategies. An innovative approach for the treatment of these lesions can be low-power light therapy (photo biomodulation), promoted by light devices such as LASER (Light Amplification by Stimulated Emission of Radiation) and/or LED (Light Emitting Diode).

Aims

The aim of this study to investigate the role of photo biomodulation (PBM) in human dermal fibroblasts for the application of wound healing. We aim to study photo bio-modulation, light treatment, as an effective and safe therapy to promote wound healing.

Methods

Photo biomodulation iCAP device was designed and made using 3D printer in the 12-well plate format with fixed 12 LED's emitting 650 nm wavelength red light. The effects of photo bio-modulation was investigated on human Dermal Fibroblasts (HDFs) with and without transforming growth factor beta (TGFβ) activation. The cells were irradiated with ~650 nm wavelength red light for 0, 80 and 600 seconds. Effects on cell viability, gene and protein expression were evaluated.

Results/ Conclusion

TGFβ induced activation of HDFs as confirmed by α-SMA, Collagen type I and type III gene expression. Photo biomodulation of 80 and 600 seconds resulted in significant downregulation of TGFβ-induced gene and protein expression of α-SMA, Collagen type I and type III as compared to the control cells. The treatment did not show any cytotoxic effects. Further mechanistic and effect studies on different cell types are currently ongoing. This study suggest PBM can be a promising approach to promote wound healing.

ABS-67514608

The supplement drug interaction between the flavonoid quercetin and alpha-blocker tamsulosin enhances vasorelaxation in vitro but not in vivo

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Quercetin is the most abundant flavonoid in fruits and vegetables and the best studied flavonoid. Epidemiological studies have revealed inverse associations between the intake of flavonoid supplements and mortality from cardiovascular diseases. It has been reported that quercetin lowers blood pressure in spontaneous

Development 2 - Method Development

hypertensive rats (SHR) [1]. Secondly quercetin increases the in vitro potency of the alpha-blocker tamsulosin to antagonize phenylephrine-dependent arterial contractions 10-fold [2].

Aims

To examine if the supplement-drug interaction between quercetin and tamsulosin luxates hypotensive and orthostatic events in vivo, several sets of studies were conducted in hypertensive (SHR) and normotensive (WKY) rats.

Methods

First, in rats pretreated with quercetin or its vehicle, (de)pressor responses to phenylephrine and tamsulosin were examined. Secondly, tamsulosin-induced changes in renal, mesenteric, hindquarter, and carotid conductance were compared in quercetin and vehicle treated rats instrumented with Doppler flow probes. Animals were also placed on a tilt table to record regional haemodynamic changes to orthostatic challenges. Thirdly, adult SHR were instrumented with telemeters to measure 24h patterns of blood pressure. Recordings were made before and during a 5 weeks oral treatment of quercetin. Finally, prehypertensive SHR were treated with quercetin from 4-8 weeks of age and arterial pressure was measured at 8 and 12 weeks.

Results / Conclusions

Pretreatment with quercetin did not influence (de)pressor responses to phenylephrine and tamsulosin, neither in WKY or SHR. While tamsulosin treatment and tilting lowered blood pressure and increased conductances in all vascular beds, effect size was not influenced by pretreatment with quercetin. Prolonged treatment with quercetin, either in prehypertensive SHR or adult SHR with established hypertension did not lower blood pressure.

Cumulatively, these data demonstrate that quercetin does not amplify haemodynamic effects of tamsulosin or tilting in vivo in rats, and has no effect on blood pressure development in SHR.

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ABS-67664800

Mechanistic CNS Modeling: From Healthy to Diseased Central Nervous System Pharmacokinetic Predictions

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CNS (central nervous system) drug development suffers a high attrition rate, particularly due to apparent drug ineffectiveness and safety concerns. Traditional methods of pharmacokinetic (PK) assessment often fail for CNS mainly due to ethical limitations of brain sampling in humans, the inaccuracy of accessible fluid sites (e.g. "lumbar" cerebrospinal fluid) concentrations as a predictor of brain (extracellular fluid) concentrations, in addition to the complexity of the CNS.

Aims

The primary goals of this research project are to further improve the previously published comprehensive CNS physiology-based PK (PBPK) model, which can predict PK profiles of a range of drugs within the CNS in healthy conditions [1], and subsequently

develop disease-specific components for CNS conditions, i.e. CNS PBPK disease models.

Methods

We started with a published and validated CNS PBPK model [1], which predicts CNS PK profiles in multiple CNS regions using the drug's physicochemical properties and CNS physiology. We extended the model with drug lipophilicity to describe brain tissue binding and Henderson-Hasselbach equations to describe drug ionization due to pH effect. To evaluate the model, the median and 95% prediction intervals of 200 simulations (including interindividual variability of the plasma PK model) were compared to previously published observed CNS unbound concentrations of 10 drugs in rat [1]. Model-based simulations were performed using R_xODE (an R-based package); plasma pharmacokinetics was modeled in NONMEM V7.3.

Results / Conclusions

The CNS PBPK model has been extended with brain tissue binding and pH effect. Model-based simulations, the resulting median, and 90% prediction intervals described the observed rat data from the different CNS compartments. With the addition of other relevant physiological processes (e.g. metabolism, active transport), this model can incorporate pathophysiological changes due to CNS diseases and thus translate to predict PK profiles in diseased populations.

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ABS-67533531

Multimodal imaging assessment of intradermal drug injection using hollow microneedles

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Background

Intradermal injection of drugs using hollow microneedles might be less painful than regular subcutaneous injection. Biological drugs which are frequently administered to children, such as adalimumab, might be candidates for this method of administration.

Aims

- 1) To determine the feasibility of intradermal injection of 40 mg adalimumab in 0.4 mL into ex vivo human skin using the MicronJet600.
- 2) To explore the usability of various imaging methods in the evaluation of intradermal drug injections.

Methods

Defatted ex vivo human skin from bariatric surgery was injected with saline with infrared dye or adalimumab. Injections were done with the commercially available hollow microneedle MicronJet600 (NanoPass, Nes Ziona, Israel). Measurements included infrared imaging for volume quantification, 3D photography for bleb size, laser speckle contrast imaging for validation (absence of perfusion), optical coherence tomography for epidermal thickness, multispectral imaging for skin colour, and thermography for skin temperature. Microneedles were inspected for damage using bright field microscopy.

Results

Single use did not damage the microneedles. The MicronJet600 could be used for intradermal injections of saline and adalimumab up to and including a volume of 0.4 mL. Bleb size of 0.4 mL



Development 2 - Method Development

injections was highly variable (mean 9.2 mm, IQ 4.7 – 13.5 mm) and distribution in the skin was generally more widespread than the bleb.

Conclusion

We obtained positive feasibility for intradermal injections with the MicronJet600 system. It can be used for injection of a volume of 0.4 mL adalimumab into ex vivo human skin. Intradermal injection can be visualized using various imaging modalities.

ABS-67572788

Novel assessment methods to explore and characterize wound healing after skin punch biopsies in healthy volunteers.

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Background

Novel models need to be developed for drug development in wound healing. These models should be feasible in execution, but also include novel biomarkers and endpoints to objectively monitor treatment effects of interventions. The gold standard, i.e. histological analysis of skin biopsies, is invasive and may induce considerable side effects. Therefore, a study in healthy volunteers was conducted to explore novel assessment methods for wound healing characterization.

Objectives

1) To evaluate imaging modalities in wound healing assessment, and 2) to compare various non-invasive skin assessment modalities.

Methods

A single-arm, observational study to characterize wound healing after three millimeter skin punch biopsies was performed in eighteen healthy volunteers. Wounds healed without secondary intervention and wound healing was assessed over 70 days using 3D imaging, transepidermal water loss (TEWL), and qPCR analysis on biopsies. Endpoints were summarized (mean, standard deviation of the mean) by time.

Results

Stereo-photogrammetric analysis showed a decrease of the wound surface after biopsy ($7.93 \pm 1.23 \text{ mm}^2$) up to day 28 when no wounds were present. TEWL baseline values ($13.6 \pm 4.3 \text{ g/m}^2\text{h}$) increased after biopsy ($62.4 \pm 8.0 \text{ g/m}^2\text{h}$) followed by a decrease, reaching steady state at day 70 ($13.6 \pm 4.3 \text{ g/m}^2\text{h}$). qPCR analysis on histology samples showed an increased protein fold compared to housekeeping gene (ABL) on day 7 for TGFB1 and VEGF-A. TGFB3 showed an increasing expression up to day 28 and decreased afterwards up to day 70.

Conclusion

Three millimeter skin punch biopsies are a suitable, minimally invasive method to study wound healing in healthy volunteers. All performed pharmacodynamic analyses showed promising results in wound healing evaluation and can be used in future intervention trials.

ABS-67573690

Comparison of non-invasive methods to quantify skin inflammation in healthy volunteers; a sub-analysis of omiganan enhancement on imiquimod-induced skin inflammation

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In a novel, rapid and reversible skin inflammation model in healthy volunteers, the effects on imiquimod (IMQ)-induced skin inflammation with and without the immunomodulator omiganan (OMI) were studied using different non-invasive methods. In a sub-analysis of the results these methods were compared for sensitivity and variability.

Aims

The objective of this sub-analysis was to find the most optimal non-invasive method to quantify skin inflammation in human. The results of this sub-analysis will be used to optimise the best read-out for future proof of pharmacology studies in (provoked) skin inflammation models.

Methods

Sixteen healthy male and female volunteers received topical IMQ, OMI or a combination of both in different sequential orders under occlusion for up to 4 days on tape stripped skin of their back. Skin inflammation was assessed daily for 5 days for signs of inflammation by different non-invasive methods including 2D photography erythema index analysis (EI), colorimetry, visual erythema grading (Clinician Erythema Assessment (CEA)) and perfusion by laser speckle contrast imaging (LSCI). Results were compared for sensitivity and variability.

Results / Conclusions

All 16 subjects completed the study. Treatments were well tolerated. Skin inflammation was clearly more apparent for the combination of IMQ with OMI 1% and 2.5% compared to IMQ+vehicle (veh) for all methods. Significant effects were demonstrated with LSCI and colorimetry, but not with EI. LSCI showed significant enhanced perfusion for IMQ+1% and 2.5% OMI compared to IMQ+veh (+14.6%, 95% CI 5.3-23%, $p < 0.01$ and +13.2%, 95% CI 3.7-21.7%, $p < 0.01$, respectively). Colorimetry demonstrated significant enhanced erythema only for IMQ+1% OMI compared to IMQ+veh (+1.5, 95% CI 0.25-2.83, $p = 0.02$). No statistical significant differences were observed with EI. For CEA, the assessment showed no clear differences between the 3 treatments. With regard to the variability, colorimetry showed less variability with a lower %CV for all 3 treatments on all days compared to both LSCI and EI (mean %CV 73%, 127%, 132% for colorimetry, LSCI and EI respectively). In conclusion, LSCI showed higher sensitivity compared to colorimetry and EI, whereas colorimetry showed a lower variability. Therefore in future studies the following techniques will be applied.

Development 2 - Method Development

ABS-66452376

Machine learning based assessment of the effect of alprazolam and alcohol on simulated driving

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The use of alcohol and sedative medicines have a negative impact on driving behavior [1]. While the assessment of driving behavior is difficult, many use the standard deviation of the lane position (SDLP) as a measure to quantify driving quality (2). The SDLP was found to be sensitive to drug induced changes, but cannot fully describe driving behavior (3). Therefore, other parameters, such as the distance to the car in front of the driver, might have additional predictive value and are expected to improve this classification.

Aims

Here, we identify which parameters obtained during (simulated) driving help in distinguishing between driving behavior resulting from the intake of placebo, alcohol, or alprazolam.

Methods

The data used in this study was obtained in the study of Huizinga et al. [2] which was a placebo-controlled, four-way crossover study with ethanol and alprazolam in 24 healthy subjects. Subjects performed a neurocognitive test, a psychomotor test, and a driving simulator test. For this analysis only the driving test was considered. The data were split into a training-set and a validation set (20 %). The training-set was again split into a training (60%) and test-set (20%). Moreover, ML was used to select features that had the highest predictive value. The accuracy of the statistical model (full model) was compared to a similar model including just the SDLP and Driving Safety Score (reduced model).

Results and conclusions

Five parameters were identified with a strong predictive value: the SDLP, standard deviation of the steer position, the average steering acceleration, gear-switching behavior, and change in speed. Moreover, models could distinguish between placebo and alprazolam (accuracy of 0.84 and 0.49 for the full and reduced model respectively), and between placebo and alcohol (accuracies were 0.64 and 0.55 for the full and reduced model respectively).

We demonstrated that addition of more parameters than SDLP improves the ability to distinguish between placebo and alcohol or alprazolam impaired driving. We observe that our model predictive performance was better for alprazolam impaired (accuracy of 0.84) than for alcohol impaired driving (accuracy of 0.64). Our data implicate that only the current standard for assessing the effect of drug treatment on driving behavior, the SDLP, is not sufficient for predicting an effect of drug treatment as shown by low accuracies.

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ABS-66451094

Optimizing I/E Criteria for Early NASH Clinical Studies with FibroScan

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Non-alcoholic steatohepatitis (NASH) is a chronic liver disease associated with cirrhosis and liver-related mortality. There is currently no approved treatment for NASH, but several drugs are in development. Liver biopsy remains the standard for disease diagnosis, but is affected by low patient acceptance, risk of complications, and non-suitability for early clinical studies. Instead, elevated serum labs such as triglycerides (TG) and alanine aminotransferase (ALT) are often used to identify populations with fatty liver or fibrosis respectively. While cut-off values for these labs are simple to incorporate into a study protocol, they may not appropriately reflect the intended study population.

Aims

To evaluate how well elevated TG and ALT predict hepatic steatosis and liver stiffness (fibrosis) respectively.

Methods

In a database interrogation study, 54 males and 79 females with BMI ≥ 30 kg/m² participated in a screening initiative to measure liver fat and stiffness with FibroScan. Measurements were coupled with clinical laboratory measurements. Liver stiffness ≥ 7 kPa and liver fat ≥ 260 dB/m cut-off values were used based on corresponding liver biopsy steatosis grade 1 and fibrosis stage 2 respectively, as described in the literature. Elevated fasting serum values were defined as TG ≥ 150 mg/dL, ALT ≥ 43 IU/L (males) or ≥ 28 IU/L (females). Moreover, commonly used NASH biomarkers and the FIB4 score were assessed. Results were analyzed in a 2x2 contingency table with positive predictive value (PPV) and likelihood ratio reported.

Results

PPV for elevated TG and liver fat was 23% and the likelihood ratio, which represents the magnitude in predictability, was small with a value of 1.3. Elevated ALT and FIB4 ≥ 1.3 had a PPV of 76% and 73% for liver stiffness respectively. Both measures also had a small effect of predicting liver stiffness with a likelihood ratio of 1.2 and 1.1 respectively.

Conclusions

Literature cut-off values for TG, ALT and FIB4 may not appropriately reflect liver steatosis or stiffness and fibrosis. FibroScan is a non-invasive screening technique and, therefore, assessment of liver fat and stiffness may be more applicable I/E criteria for early clinical NASH studies than traditional labs alone.

ABS-66451217

Assay Validation and Clinical Performance of Chronic Inflammatory and Chemokine Biomarkers of NASH Fibrosis

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Development 2 - Method ...

Non-alcoholic steatohepatitis (NASH) is a chronic liver disease that can lead to cirrhosis, liver transplant, and even hepatocellular carcinoma. While liver biopsy remains the reference standard for disease diagnosis, development of non-invasive soluble biomarkers of NASH are of great importance for early detection.

Aims

Analytical and clinical development of non-invasive soluble biomarkers of NASH fibrosis.

Methods

Analytical and clinical validation was done on a series of pro-inflammatory cytokines and chemokines implicated in hepatic inflammation; interleukin-6 (IL-6), C-reactive protein (CRP), tumor necrosis factor alpha (TNF α), macrophage chemoattractant protein 1 (MCP-1), macrophage inflammation protein 1 beta (MIP-1 β), eotaxin, vascular cell adhesion molecule 1 (VCAM-1). Biomarker assays were validated for accuracy and precision. Clinical performance was evaluated in a random sample of 52 patients with biopsy-proven non-alcoholic fatty liver disease (NAFLD)/NASH. Patients were categorized according to their fibrosis stage into; advanced (F3-F4), mild (F1-2) and no (F0) fibrosis.

Results

Serum IL-6 was increased in patients with advanced fibrosis (2.71 pg/mL; 1.26 pg/mL; 1.39 pg/mL $p < 0.01$) compared to patients with mild or no fibrosis respectively. While, there was no significant difference noted in CRP, TNF α , MCP-1, MIP-1 β , eotaxin among the three groups, VCAM-1 levels were increased by 55% ($p < 0.01$) and 40% ($p < 0.05$) in the advanced cohort compared to the mild and no fibrosis groups respectively. VCAM-1 also displayed good clinical performance as a biomarker of advanced fibrosis with an area under the receiver operating curve of 0.87.

Conclusions

The VCAM-1 assay demonstrated robust accuracy and precision, and VCAM-1 outperformed IL-6, CRP, TNF α and the chemokines MCP-1, MIP-1 β , and eotaxin as a biomarker of advanced fibrosis in NASH. Addition of biomarkers such as IL-6 and VCAM-1 to panels may yield increased sensitivity and specificity for staging of NASH.

Discovery 1 - Cell and ...

ABS-64088919

Synthesize, characterization and evaluation cytotoxicity and nephrotoxicity of three metal complexes of Disodium 5,5'-bistetrazole

Complexes based on heavy metals can be used for the treatment of a wide variety of cancers but their effectiveness and toxicity depends on the metals and ligands used. The aim of this project was to synthesize three new complexes and evaluate their anti-cancer and nephrotoxic potential in vitro. To this end, we synthesized 5-substituted tetrazole salt, disodium 5,5'-bistetrazole, 5,5'-Na₂BT and reacted this compound with three different metal salts; KOH, Pb(NO₃)₂·3H₂O and Cd(CH₃COOH)₂·2H₂O which resulted in K(BT)(CH₃OH), {1}, {Pb(BT)₃·(H₂O)₃}, {2} and Cd(BT), {3}. The crystal structure of complex {1} and {2} were also reported. The structure of complex {3} was characterized by LC/MS, ¹³C-NMR and CHN elemental analysis. We also evaluated cytotoxicity of 5,5'-Na₂BT salt and its metal complexes in Breast cancer cell line (MCF-7) and compared to that of cisplatin. Induction of apoptosis in MCF-7 cells exposed to Cd(BT), Real Time-PCR and western blot analyzes. Finally, conditionally immortalized human proximal tubule epithelial cells, ciPTEC cell line was used for assessing cytotoxicity potential of Cd(BT) in non-cancerous kidney cell line.

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How effective may be virtual screening in identifying c-src tyrosin kinase inhibitors?

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Src tyrosin kinase inhibitors are currently explored as potential new therapies in a variety of malignancies, as well as for other pathologies, such as certain neurodegenerative diseases or pulmonary hypertension. Whereas the classical drug development process tended to be costly and tedious, computational methods have high efficiency and are rather inexpensive.

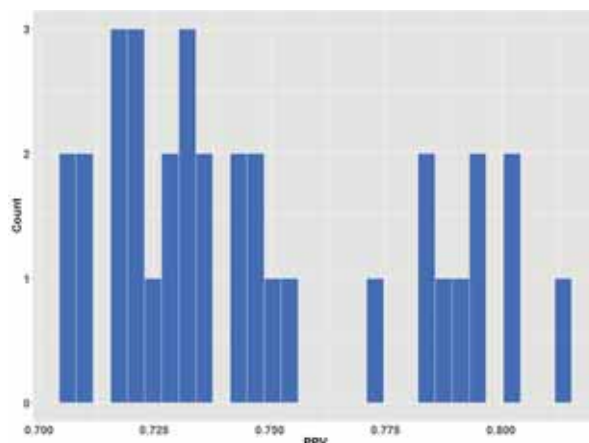
Aims

Our main objective was to identify new inhibitor ligands for c-src tyrosin kinases using virtual screening, by means of multiple validated QSAR models based on classification machine learning algorithms, with a variety of descriptor sets, assembled by stacking.

Methods

The data set was collected from ChEMBL and consisted of 1151 k_i values for c-src tyrosin kinase for 1038 chemical compounds (for duplicate values the average k_i was used). Compounds with $k_i < 100$ nM were classified as "active", whereas those with $k_i > 100$ nM as "inactive". Chemical descriptors (2D) were computed using Dragon 7.0. All models were developed in R, using 16 different algorithms for feature selection and nested cross-validation for the evaluation of performance. The following algorithms were employed: random forests, support vector machines, AdaBoost, Bayesian additive regression trees, binomial regression, k-nearest neighbors, and C5.0. Three different approaches were explored for the applicability domain of the models. The models were stacked and applied for the virtual screening of a ZINC data set (named compounds) containing over 104000 chemical substances.

Discovery 1 - Cell and Signaling



Results / Conclusions

34 models with good performance (specificity higher than 90%, positive predictive power (PPV) higher than 70%) were used for virtual screening. 1054 compounds were identified with an estimated PPV of 80.95%. Indirect evidence from the literature indicates that these predictions are reliable.

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ABS-66327631

Predicting Polypharmacology in Kinases

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Introduction

The term polypharmacology is used when drugs interact with multiple protein targets. Conversely there is selectivity, in which compounds are designed to target only one protein. The design of multi-target drugs is gaining attention to tackle more complex disease mechanisms, such as inflammation and cancer.¹ The design of polypharmacological compounds is fueled by two main reasons. Firstly, previous efforts show that selective kinase inhibitors become ineffective over time as a result of drug resistance.² Secondly, multi-drug treatments are not ideal as drug-drug interactions may occur. Therefore, polypharmacological drugs are of interest when targeting multiple kinases simultaneously, as it is hypothesized that they reduce the onset of resistance.²

Objectives

In the light of the Multi-Targeting Drug DREAM challenge³ we developed a strategy to predict compound activity profiles for kinases. In the cancer case study, polypharmacology modeling was limited to nine pre-defined kinases. These kinases were subjected to three successive modeling techniques to assess 'activity' of compounds: statistical modeling, structure-based modeling, and molecular dynamics simulations. Ultimately we virtually screened the ZINC database⁴ for compounds that adhered to our desired pharmacological profile.

Methods

We utilized statistical models that were trained and validated on public data derived from ExCAPE⁵ and ChEMBL⁶. These models were used to filter the screening compounds and promising compounds succeeded to the structure-based phase. In structure-based modeling we first thoroughly benchmarked all available protein crystal structures of the kinases at hand using docking. SPLIF^{7,8} scores were generated for compounds in all protein structures. The multiple docking and SPLIF scores per compound for each target were combined using Z2 scoring⁹. The resulting ensemble models for each target had high (early) enrichment scores indicating that these models are predictive.

The most promising compounds were re-scored with molecular dynamics using pose-metadynamics.¹⁰ Binding pose metadynamics enhanced compound ranking when tested on sets of 100 compounds per target.

Results and Conclusion

From the resulting compounds we selected 50 compounds that fitted the desired polypharmacology profile. Although for this case study we utilized our strategy for a given set of kinases, this method can easily be applied to any target family, provided that sufficient data is available.

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ABS-66452855

Development of a 3D model to study the fibro-inflammatory responses and macrophage infiltration cued by liver stiffness

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Discovery 1 - Cell and Signaling

Introduction

The progression of chronic liver diseases is characterized by an excessive extracellular matrix (ECM) deposition and inflammation (1). In-vitro disease models of such chronic diseases are useful in understanding the disease pathogenesis and provide a drug screening platform for the development of personalized treatments (2). 3D models have gained prominence in recent years over traditional 2D culture platforms as they provide a biomimetic 3D microenvironment recapitulating the disease physiology (3).

Aims

The aim of this study is to mimic the fibrotic liver environment by constructing decellularized ECM (dECM)-based 3D-liver lobules with varying stiffness. This platform is then employed to investigate how fibrosis-driven liver stiffness cues the fibro-inflammatory responses and macrophage infiltration.

Methods

Porcine liver was decellularized by perfusion with detergents using a standardized protocol and was subsequently lyophilized. The freeze-dried a-cellular tissue was ground using a rotary knife mill and digested in 0.5M acetic acid. Digested dECM was mixed with collagen (0.5% w/v) in different ratios and incubated at 37°C to form gels of varied stiffness. Human HSCs (LX2 cells) were cultured in these gels with different stiffness and tested for cell viability using live/dead assay.

Results / Conclusions

Decellularization of the liver was effective and the absence of cells was confirmed by H&E and fluorescent nuclear staining. No cellular DNA residues were detected. The decellularization procedure preserved ECM matrix as shown by immunostainings for collagen-I. Gels of concentrations 0.5%, 1% and 2% (w/v) were formed from dECM in 3 to 4 hours. Mixed collagen-I/dECM gels were developed in ratios of 1:3, 1:6, 1:12 to have different ECM stiffness. These mixed gels are currently characterized using Scanning Electron Microscopy (SEM), rheological measurements, and will be recellularized with macrophages and co-cultures of various liver cell types for evaluating fibro-inflammatory responses using specific markers by immunostaining's and qPCR. In conclusion, in this study we developed a 3D model to study fibrosis by mimicking mechanical stiffness. This model will be employed to improve our current understanding of liver disease pathogenesis and will fasten the development of personalized medicine approaches.

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ABS-66124814

Positive effects of small molecule tryptophan-2,3-dioxygenase inhibitors on motor and non-motor symptoms in a murine model of Parkinson's Disease

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In the brain around 95% of the amino acid L-tryptophan is metabolized by enzymes of the kynurenine pathway. Parkinson's disease (PD) is associated with an imbalance between neurotoxic (3-hydroxykynurenine and quinolinic acid) and neuro-protective (kynurenic acid) kynurenine pathway metabolites.¹ Tryptophan-2,3-dioxygenase (TDO) catalyses the first, rate-limiting step of the kynurenine pathway and was identified as a potential target for PD in a genome-wide RNA interference screen in a *Caenorhabditis elegans* model for protein misfolding.² To investigate TDO inhibition as a potential therapeutic strategy for PD, a selective small molecule TDO inhibitor was developed by high-throughput screening and subsequent optimization by medicinal chemistry.³

Here we describe the effect of TDO inhibition by the selective small molecule TDO inhibitor NTRC 3531-0⁴ on the CNS and intestinal phenotypes of a rotenone-induced murine model for PD. The structurally unrelated TDO reference inhibitor 680C91, published by the Ludwig Cancer Center,⁵ was tested in parallel. Mice were injected with rotenone or vehicle in the striatum. The treatment (NTRC 3531-0: 25, 50 & 100 mg/kg and 680C91: 12.5, 25 & 50 mg/kg po, QD) started 7 days after disease induction. Motor and cognitive functions were tested, intestinal transit was analysed and histological examination of the brain and the colon was performed.

Treatment with NTRC 3531-0 at 50 and 100 mg/kg and with 680C91 at 50 mg/kg significantly improved motor function in PD mice in comparison to untreated mice. At the highest dose both inhibitors also improved cognitive function. Moreover, treatment with TDO inhibitors resulted in less dopaminergic cell loss in the substantia nigra in PD mice. Rotenone impairs intestinal transit in mice, which reproduces constipation in human PD patients. TDO inhibition improved intestinal transit in PD mice. In addition, both inhibitors reduced gross colonic inflammation, glial cells activation and enteric neuronal α -synuclein accumulation in PD mice.

Taken together, this study indicates that modulation of the kynurenine pathway through inhibition of TDO with small molecular compounds might be a potential therapeutic strategy for PD.

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Discovery 1 - Cell and Signaling

ABS-67692005

Towards robust quantification of cytokine expression in complex body fluids and organ tissue

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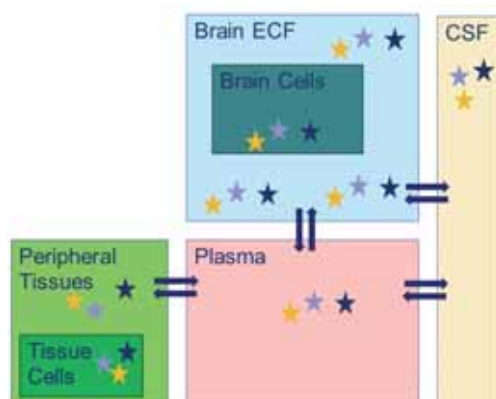
Cytokines are glycoproteins produced upon infection and tissue damage, they are produced locally and distributed within the blood and local tissue environment¹. Cytokines are regulators of the immune system, making them informative biomarkers for inflammation and inflammatory diseases². Prediction of the inflammatory state in the organ of interest based on measurements in performed solely in blood requires prior knowledge on the interdependencies of distribution between different body compartments. To unravel these interdependencies robust absolute quantification of proteins of interest in both organs and body fluids is needed. Protein multiplexing is a sensitive ELISA-based technique, enabling the detection of multiple cytokines at the same time in the same sample.

Aims

The aim of this study is to robustly quantify absolute protein levels including cytokines and chemokines in complex body fluids and a variety of organ tissues, to be able to directly compare distribution of cytokines over different body compartments.

Methods

Body fluids and organs harvested from healthy wild type rats are used as starting material. Homogenization of organ tissue is performed using the bullet blender tissue homogenizer. Different dilutions of body fluids and organ homogenates are subjected to multiplex assays for the detection of cytokine and chemokines.



Overview of the different body compartments and their interdependencies affecting cytokine levels. Stars represent chemokines, and pro- and anti-inflammatory cytokines, ECF: extracellular fluid, CSF: cerebrospinal fluid.

Results / Conclusions

Even though the multiplex assay shows high reproducibility over a large dynamic range (1 pg/ml to 10.000 pg/ml), dilution dependent artifacts arise when measuring in complex matrices. Depending on the cytokine of interest the linear range is strongly reduced either due to saturation-dependent effects caused by inhibitory proteins present in the matrix, or by a-specific binding of proteins present in the matrix, indicating an intricate balance between strong dilution of sample and matrix components while obtaining high reproducibility of individual measurements.

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ABS-67713452

Elucidating the prerequisites of forming the allosteric pocket in ROR γ t

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Introduction

The retinoic acid receptor-related orphan receptor- γ t (ROR γ t) is one of 48 members of the human nuclear receptor (NR) superfamily. Within this family, there is a high level of homology and all members have a similar domain structure. The ligand binding domain (LBD), which upon compound binding can induce an altered stability and resulting from that cofactor recruitment, is especially highly conserved. This presents the challenge of specifically targeting one NR.

Objectives

In 2015, an allosteric binding pocket in ROR γ t was discovered. Despite current efforts in engineering compounds binding ROR γ t allosterically, the mechanism underlying the formation of this pocket is poorly understood. To elucidate if the pocket is a unique asset of ROR γ t or could also be formed in other NRs we aim to identify the prerequisites for the allosteric pocket.

Methods

Key-differences between ROR γ t and other NRs in the allosteric region were identified. A unique feature within the ROR family is the presence of a helix 11 prime (H11') connecting H11 and H12, which, upon allosteric compound binding, unfolds and spans over the pocket. H11' in ROR γ t was shortened in order to determine if NRs with a shorter linker could be able to form the allosteric pocket. Further, residues in direct contact with the ligand or connecting distant helices in the allosteric fold were pinpointed. Wildtype ROR γ t LBD was mutated at these locations (Figure) and the mutants were tested in biochemical assays.

Results

Upon compound titration in an HTRF assay, all mutants showed similar IC₅₀ values for orthosteric (inverse) agonists as the wildtype protein. The IC₅₀ value of the wildtype protein in response to allosteric inverse agonist was 12.1±0.5 nM. W317F, Q329A and Q484A still had nanomolar IC₅₀ values, while Q487K and the deletion mutants had IC₅₀ values in the micromolar range.

Conclusion

Based on our findings, it can be concluded that Q487 and a H11' of at least nine amino acids are



Figure: ROR γ t LBD with allosteric inverse agonist (PDB: 4YPO). Mutated residues are indicated.

Discovery 1 - Cell and Signaling

crucial for the allosteric binding pocket. ROR α and ROR β , the only NRs with a nine residue H11', both have a lysine at 487 position. Therefore, it can be concluded that no other NRs would be able to form this exact same pocket.

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ABS-66439132

2'-Fucosyllactose modulates phenotype and function of dendritic cells depending on the presence of LPS

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Specific human milk oligosaccharides (HMOs), like 2'-Fucosyllactose (2'-FL), are now available for use in food products. HMOs have been suggested to directly interact with human immune cells and elicit immune effects¹. Are those effects mediated through the HMOs or by low level of side products present in HMOs?

Aims

We aim to compare biotechnologically produced 2'-FL (b2'-FL) and chemically synthesized 2'-FL (s2'-FL) versus purified b2'-FL (p2'-FL) on dendritic cells (DCs) phenotype and function. Also, we aimed to determine whether 2'-FL can initiate immune effects by itself or an additional immune trigger is necessary.

Methods

Human and mouse DCs were treated with different concentrations of b2'-FL, s2'-FL or p2'-FL with or without LPS for 24h. DCs were analysed for phenotypic changes by flow cytometry, and cell supernatant was collected for cytokine analysis by ELISA. Also, 2'-FL -treated mouse DCs were co-cultured with naive CD4+ T-cells to investigate effects on T-cell polarization.

Results / Conclusions

2'-FL alone did not show effects on DC phenotype or function. In the presence of LPS, b2'-FL but not p2'-FL, dose-dependently induced increased levels of IL-10, IL-12p70, IL-6 and TNF- α —suggests an important role for the side products present in b2'-FL. On murine DCs, s2'-FL and p2'-FL induced LPS-dependent lower expression of MHC-II but higher expression of co-stimulatory markers. Moreover, both 2'-FLs increased IL-10 and IL-27 production by murine DCs, as well as increased IL-10 producing Foxp3+Tregs from naive CD4+ T-cells in the mixed lymphocyte reaction.

Our data indicate that (1) 2'-FL needs an additional immune trigger to exert immune modulation on both human and mouse DCs; (2) 2'-FL in combination with LPS (immune trigger) is capable of inducing tolerogenic mouse DCs; (3) Human and mouse DCs react differently to 2'-FL.

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Critical assessment of yield and variability in isolation and characterization of extracellular vesicles from human plasma using different methodologies

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Extracellular vesicles (EVs) are functional nano-sized particles secreted by different cells of body, which contain specific molecular information of their parent cells. EV based biomarkers holds great potential in disease diagnosis, however, the isolation methods and detection protocols for EVs are highly variable from different research groups especially for plasma samples. Different EV isolation methodology may have great impact on the biomarker profile discovered in downstream analysis.

Aims

In this study we aim to investigate different EV isolations from pooled human plasma using different characterization technologies to display the feature of EV yields, the variability, as well as the reproducibility of these isolation methods.

Methods

Three different commercially available EV isolation kits (Exoquick® System Biosciences, Exo-spin® Cell Guidance Systems and qEV® iZON science) have been applied to isolate EVs from pooled plasma of 6 healthy volunteers and concentrated SK-N-SH cell derived culture media (HansaBiomed) was used as the positive control of the EV containing matrix. Nanoparticle tracking analysis (NTA) was used to measure the particle concentration and size distribution of the isolates. Western blotting and Wes (ProteinSimple) were used to characterize specific protein markers in the isolates (CD9, CD63, and Flotillin-1). EVQuant (Erasmus MC) was used to measure the quantity of general vesicles and immune-labeled EVs. Transmission electron microscopy (TEM) was used to characterize the morphology of EVs in isolates from different isolations.

Results

A double peak pattern for the particle size distribution of EV plasma isolates was found from each method in NTA, where the second peak (175nm – 250nm) matches the size of EVs. In protein characterizations CD9 bands (20-25 kDa) were found in qEV fraction 1 and 2, in Exoquick and in Exo-spin samples, but not being reproducible. Different from the positive control EV sample, a CD9 antibody specific band at 45-50 kDa were repeatedly found in plasma Exoquick and Exo-spin isolates, which have not been reported previously. Flotillin-1 bands (49 kDa) were observed except for qEV fraction 3. CD63 bands (50-55 kDa) were found specific to Exo-spin samples and qEV isolation fraction 3. TEM also showed different patterns of morphology between EVs from different isolation methods.

Conclusion

Different isolation methods tend to enrich certain subpopulation of EVs, while there was no isolation method that in which the isolates included all the tested EV protein markers. This is an important finding, as it could impact the downstream EV based biomarker analysis and conclusions drawn on that basis.

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Affinity-based protein profiling of the adenosine A1 receptor

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The adenosine A1 receptor (A₁R) is a G protein-coupled receptor that plays a role in many physiological and pathological processes, such as lipolysis, ischemia and reperfusion injury.^{1,2} Extensive chemical and pharmacological studies have already resulted in various potent ligands for the A₁R. However, due to unwanted side-effects, no A₁R ligand has thus far reached the markets. It is therefore important to get a better understanding of the exact functioning of the A₁R in various tissue types. New chemical tools are required to generate a comprehensive depiction of all the ligand-induced effects on a molecular level.

Aims

This study focuses on the synthesis, pharmacological evaluation and application of affinity-based probes (AfBPs) for the A₁R. AfBPs are chemical tools that consist of an electrophilic reactive group ('warhead'), that is able to covalently bind to a protein, a label ('reporter tag'), that allows for the detection of protein molecules after the probe has bound and a recognition element ('scaffold'), to implement selectivity towards a protein or class of proteins. Using AfBPs, a biochemical platform will be created to study receptor expression, localization and target-engagement of the A₁R, as well as determination of possible off-targets of known A₁R ligands.

Methods

Synthetic organic chemistry has been used to synthesize a small panel of AfBPs. These AfBPs were evaluated through SDS-PAGE and radio-ligand binding experiments, making use of membranes isolated from CHO cells overexpressing the A₁R.

Results / Conclusions

Our group has recently synthesized LUF7746, a covalent ligand for the adenosine A1 receptor. Based on this ligand, various AfBPs were synthesized, differing in the position and orientation of the alkyne group. This alkyne group allows the probe-receptor complex to be 'clicked' to various azide-containing reporter groups in a biorthogonal click reaction, allowing the receptor to be detected in biochemical assays. The first compounds within our small subset of affinity-based probes have already been evaluated through gel-based and radio-ligand binding assays. More experiments are currently being performed to confirm the labeling properties of these affinity-based probes.

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CXCR4-targeting nanobodies as modulators of receptor dimerization and function

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Introduction

Emerging evidence indicates the importance of receptor oligomerization for the signal transduction by transmembrane receptors, including chemokine receptors belonging to the class A GPCRs such as CXCR4¹. Considering the prominent role of CXCL12/CXCR4 signaling in disease (e.g. cancer and HIV) and the ability of CXCR4 to form constitutive and ligand-induced homodimers^{1,2}, the molecular mechanisms and functional consequences of oligomerization processes largely remain elusive to date.

Aims

Elucidating the impact of dimerization on CXCR4 function using CXCR4-targeting nanobodies.

Methods

Use of CXCR4-targeting nanobodies with distinct epitopes to modulate the dimerization state of CXCR4 and evaluate the impact of this on downstream signal transduction pathways using BRET biosensors.

Results / Conclusions

Monovalent and Fc-linked CXCR4-targeting nanobodies with distinct epitopes³ promoted or attenuated the basal dimerization state of CXCR4. The altered oligomerization state resulted in modulation of dimerization-dependent signaling downstream of this receptor. Moreover, a subset of the nanobodies showed inverse agonistic properties at the constitutively active mutant of CXCR4. Our results demonstrate the importance of CXCR4 dimerization for the signal transduction of this receptor with the nanobodies being interesting tools to further elucidate the mechanism underlying this process.

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Identifying high affinity ligands for the Plasma Membrane Monoamine Transporter using proteochemometrics

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Solute Carriers (SLCs) are a relatively low investigated receptor family, compared to other major receptor groups such as kinases and G Protein-Coupled Receptors. However, lately a drive to focus on this group was started, with increasingly more research focused on this group of transporters. Several consortiums, such as RESOLUTE, combine research groups to investigate SLCs, as they may have major effects on important pathways such as oncogenesis.^{1,2}

One of these SLCs is the Plasma Membrane Monoamine Transporter (PMAT). PMAT (SLC29A4) is a low-affinity, high-capacity transporter for monoamine neurotransmitters such as serotonin and dopamine, and is a member of the equilibrative nucleoside transporters (ENT). PMAT also functions as an



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efflux transporter for various cationic drugs and neurotoxins in the brain. While the exact mechanisms aren't clear yet, PMAT has been related to nephrotoxicity and brain damage.^{3,4} However, PMAT has also been linked to preventing ischemia or as a possible cancer treatment, which makes it interesting as a possible drug target.^{1,5}

The 3D structure of PMAT is not known and there is not a good assay available to test effects on this SLC. However, three inhibitors were found that affect the PMAT: Decynium-22, Lopinavir and TC-T6000. There are some differences in selectivity, where mainly Decynium-22 is potent on PMAT, but also affects organic cation transporters (OCT1-3).⁶ Lopinavir is less potent, but very selective for PMAT, and TC-T6000 which falls between the former two.⁷

The goal of this study was to find structures similar to Decynium-22, that were high affinity but more selective. Using the three inhibitors and the known data for these inhibitors, as well as structural information for PMAT, the ENT and OCT families, QSAR models were made. These models were used to predict new (untested) small molecules that could be tested experimentally in further research. These models were even further optimized with a forward backwards feature selection and parameter optimization. A selection was made and several predicted small molecules were presented as high-affinity, PMAT inhibitors.

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Binding kinetics of the long acting H1R antihistamine bilastine

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Aims

In the body, concentrations of (endogenous) ligands are dynamic due to processes like absorption, distribution, metabolism and excretion (ADME). This dynamic nature makes it hard to predict the effect of an antihistamine based on just its affinity constant,

which only accurately predicts receptor occupancy under stable (equilibrium) conditions. The dissociation rate predicts how long a ligand resides on the receptor (residence time), which is considered to be an important metric for drug effectiveness. One of the ways in which long residence time drugs are thought to excel, is by their long duration of action, which by residing on the receptor can exceed the bioavailability in the blood.¹ Bilastine is an antihistamine with an excellent drug safety profile and a long duration of action in vivo.^{2,3} Here we reverse engineer the molecular pharmacology of bilastine, which based on the long duration of action is suspected to be one of a long residence time inhibitor.

Methods

Radioligand binding experiments were employed to characterize the binding of unlabeled ligands at the histamine H₁ receptor (H₁R).⁴ The histamine-H1R mediated intracellular calcium levels were measured as was reported in Bosma et al. 2017.⁵

Results / Conclusions⁶

It is shown that bilastine (pK_i = 8.1 ± 0.1) has a good binding affinity for the H₁R which was similar to diphenhydramine (pK_i = 8.1 ± 0.1). Despite this equal affinity, the residence time of bilastine (RT = 73 ± 5 min) on the H₁R exceeded that of diphenhydramine (RT = 0.41 ± 0.09) by more than 100-fold and was comparable to the long residence time antihistamine fexofenadine (RT = 60 ± 20). It was shown in vitro, that bilastine and fexofenadine retained their inhibitory properties for more than an hour after washing away all unbound ligands, whilst inhibition by diphenhydramine was immediately reversed after washout. This suggests that residence time is a good metric for predicting the long duration of action by H₁R antihistamines in vivo.

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Novel affinity-based chemical probes for the C-C chemokine receptor CCR2

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The CCR2 receptor is a G protein-coupled receptor (GPCR) that, together with its ligand CCL2, has been found to play a distinct role in a number of diseases, including cancer[1-3]. Despite the clinical significance of this signaling axis, however, ligands aimed at interfering with CCR2 function have shown disappointing results in clinical trials. To address this issue, we have become interested in providing a novel means of studying CCR2 in its native environment through the use of novel chemical probes.

Aims

In the present study we aimed to develop highly versatile affinity-based probes for the CCR2 receptor. When bound to the receptor, these probes can undergo ligation to reporter groups through “click” coupling. These chemical probes will be applied in the two-step labelling of CCR2 after which the resulting probe-receptor complex will be visualized using SDS-PAGE electrophoresis and Western Blot. These experiments will

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be supported by competition binding experiments and washout assays.

Methods

Starting from the reversible allosteric antagonist SD-24, we report the development of an allosteric covalent inhibitor; LUF7591. We show that the binding affinity of this compound to CCR2 is time dependent in nature, and that its binding is resistant to multiple washout steps. Using LUF7591 as a template, we then looked to introduce a series of different electrophilic warheads, as well as a terminal alkyne which supports “click” ligation to an azide-bearing fluorescent dye (see figure).

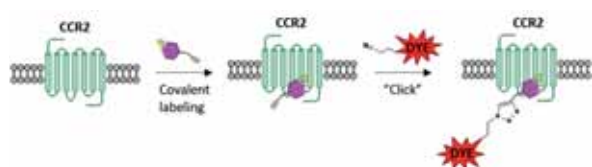


Figure. Schematic representation of the two-step labelling of CCR2.

Results / Conclusions

A series of SD-24 derived compounds were synthesized containing acrylamide – and thiocyanate warheads and linkers of varying lengths bearing a terminal alkyne. Competition binding experiments revealed that despite the structural modification of SD-24, these compounds retain nanomolar affinity. A time-dependent shift in affinity furthermore suggests these compounds bind to the receptor in a covalent manner, further encouraging their use in affinity-based proteomics. Finally, in SDS-PAGE experiments we were able to show fluorescent labelling of CCR2. Ultimately, these probes have the potential to be used in a range of in vitro and in vivo studies, allowing the study of CCR2 function in its native environment.

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SK channel activation potentiates auranofin-induced cell death in glio- and neuroblastoma cells

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Brain tumours are among the most deadly tumours being highly resistant to currently available therapies. The proliferative behaviour of gliomas is strongly influenced by ion channel activity. Small-conductance calcium-activated potassium (SK/KCa) channels are a family of ion channels that is associated with

cell proliferation and cell survival [1,2]. A combined treatment of classical anti-cancer agents and pharmacological SK channel modulators has not been addressed yet.

Aims

The aim of our study was to study cancer cell death with the gold-derivative auranofin targeting thioredoxin reductases in combination with CyPPA to activate SK channels in neuro- and glioblastoma cells.

Methods

Effects on cell death and metabolism were studied in neuroblastoma (SK-N-AS), glioblastoma (U251) compared with neuronal (HT22) cells. In addition, spheroids were generated from U251 cells to study invasion. Furthermore, cancer stem cell derived 3D neurospheres were generated from patient-derived glioblastoma GG16 cells to study cell death and to observe morphological changes caused by auranofin + SK channel activation.

Results / Conclusions

Auranofin induces cell death in a concentration-dependent manner in both neuronal and tumour cells, by affecting reactive oxygen species (ROS) generation, mitochondrial integrity and respiration. Combined treatment with auranofin and CyPPA induced massive mitochondrial damage and potentiated auranofin-induced toxicity in neuroblastoma cells, while neuronal cells are less affected by the combined treatment. These findings were recapitulated in patient-derived glioblastoma neurospheres. Taken together, integrating SK channel openers to affect mitochondrial health was identified as a promising strategy to increase the effectiveness of anti-cancer agents and potentially overcome resistance.

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Updated DrugEx: Drug Molecule De Novo Design by Multi-Objective Reinforcement Learning for Polypharmacology - A case for the Adenosine Receptors

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During this decade deep learning has progressed tremendously in both image recognition and natural language processing. Now it is increasingly applied to other data rich fields [1]. In drug discovery recurrent neural networks (RNNs) have been shown to be an effective method to generate molecular libraries [2, 3]. We published a new method named DrugEx that integrates an exploration strategy into RNN-based reinforcement learning to improve the diversity of the generated molecules [4].

Aim

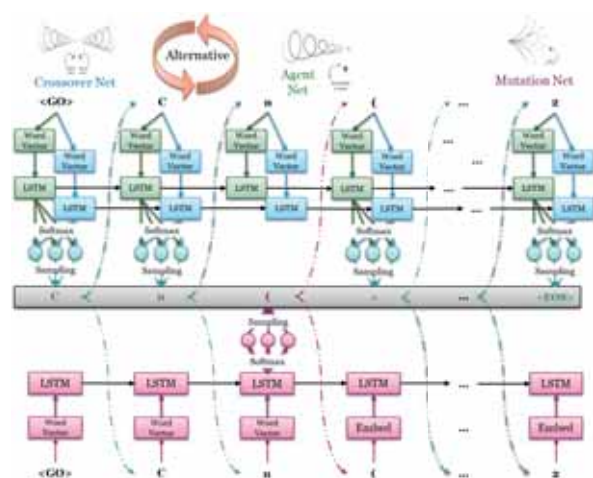
Most current deep learning-based methods focus on a single target to generate drug-like active molecules. However, drug molecules often interact with more than one target for

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better (polypharmacology) or for worse (side effects). In this perspective, we extended our DrugEx algorithm with multi-objective optimization and generated drug molecules against more than one specific target (four adenosine receptors, A_1 , A_{2A} , A_{2B} , and A_3 , in this study).

Methods

In our model, there were an agent and an environment that interplayed with each other under the reinforcement learning framework. We applied an RNN as the agent and machine learning predictors as the environment, both of which were pre-trained before in advance. During the training loop, the Agent generated a batch of SMILES based molecules. Subsequently the reward was provided by the environment, which was calculated by the weighted sum of these target prediction scores. The agent was trained under the guidance of the reward to make sure it could generate more desired molecules after convergence of the training process.



Conclusions

Our proof of concept generated compounds with a diverse predicted selectivity profile toward multiple targets, offering the potential of high efficacy and lower toxicity.

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Identification of the histamine H4 receptor interactome

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G protein-coupled receptors (GPCRs) play an essential role in the regulation of cell function by activating G protein- and/or β -arrestin-mediated intracellular signaling upon binding of their cognate ligands, and are consequently important targets for therapeutic intervention. The last couple of years it became

apparent that GPCRs can interact with other non-canonical proteins that may modulate GPCR functions. These so-called GPCR interacting proteins (GIPs) are hypothesized to modulate or even initiate distinct biochemical signaling in a more cell type-specific manner. We recently identified novel GIPs that constitutively interact with H4R using a split-ubiquitin membrane yeast two-hybrid screen (SUMY2H) on a T lymphocyte cDNA library under basal conditions. Interestingly, stimulation of the H4R with histamine decreased this interaction. We validated these ligand-modulated interactions in HEK293T cells by using bioluminescence resonance energy transfer (BRET) and enzyme-fragment complementation (EFC)-based assays to dynamically monitor their proximity, and subsequently aim to determine the functional consequences of these H₄R-GIP interactions.

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Development of a physiologically relevant in vitro 3D model of hepatocellular carcinoma for drug development

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Introduction

Hepatocellular carcinoma (HCC) [1] is a very aggressive type of liver cancer and a growing cause of cancer-related mortality worldwide. To date, there is no effective treatment against HCC, and drug development is hindered by a lack of suitable models that can recapitulate the physiology and complexity of this cancer. Altogether, there is an unmet need to develop new strategies and models to accelerate drug development.

Aims

The aim of this study is to establish a physiologically relevant in vitro platform for drug development against HCC. Our approach consists of engineering a liver micro-tissue that recapitulate key-features of the tumor microenvironment such as the characteristic extracellular matrix (ECM), vascularization, immune cells infiltration and polarization, that are responsible for dictating cancer progression and its response to therapy.

Methods

Human liver cell lines were assembled in different ratios into liver multicellular tumor spheroids (MCTS) using a high-throughput micro-well array (MWA) [2], using round bottom microwells to improve tissue morphology. Spheroids were characterized in terms of growth, stability, morphology and viability over time. Cancer-like features were investigated by gene and protein expression analysis, respectively, by qPCR and immunostaining of cryo-sections as well as whole spheroid after tissue clearing.

Results & Conclusion

Using the MWA platform, compact 400- μ m-sized spheroids were formed that remained viable for two weeks. Gene expression revealed upregulation of ECM markers, activation of proliferative and anti-apoptotic pathways, epithelial-to-mesenchymal transition, and presence of biomarkers related to poor prognosis or relapse, which are the hallmarks of cancer. Through collagen I immunostaining, collagen fibers were found to form a capsule around the carcinoma, a characteristic histological feature of HCC. Pre-vascularization of the liver spheroids was achieved by incorporating endothelial cells during spheroid formation. We are currently optimizing this vascular network and its connection

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to larger blood vessels in a microfluidic device, which is an essential feature of the tumor microenvironment and for delivery of therapeutics to the tumor. We envision that this technology would serve as a tool to improve our current understanding of cancer physiology and fasten drug development.

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Characterisation of the signalling properties of the HCMV-encoded receptor UL78

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HCMV is a member of the β -herpesvirus family and widely spread in a large percentage of the population¹. The genome of HCMV encodes four viral GPCRs namely US28, US27, UL33, and UL78. US28 is activated by various chemokines and activates various inflammatory and proliferative signalling pathways in a constitutively active manner². In contrast to US28, UL78 is considered an orphan receptor since no ligands were identified to bind this receptor yet. The UL78 like genes are present in all β -herpesvirus and are highly conserved in clinical isolates³, yet little is known about the UL78 signalling properties.

Aims

The aim of this study is to determine the signalling properties of UL78, whether it is able to constitutively activate various signalling pathways. Insight into this is important to attribute a potential role of this receptor in HCMV associated diseases.

Methods

DNA constructs of HA tagged UL78 and C-terminal mutant of UL78 receptor were amplified via PCR, the DNA was inserted to pcDEF3 vector plasmid. All DNA constructs were verified by sequencing. HEK 293T cells were transiently transfected with UL78 WT, HA-UL78, and UL78-HA, the expression of the receptors were detected by immunofluorescence and ELISA stained with anti HA and anti UL78 antibody. Reporter gene assays were performed in transiently transfected HEK 293T cells with 100 ng/well of receptor (UL78 WT, HA-UL78, and UL78-HA) and 1000 ng/well of reporter plasmid. An additional plate was prepared for ELISA to control receptor expression level.

Result / Conclusion

Immunofluorescence assay and ELISA showed that all DNA construct of UL78 are expressed in transiently transfected HEK293T cells and the expression was in dose dependent manner. Several reporter gene assays were performed to determine signalling activity of UL78 and UL78 marginally activated NFAT and NF-kb reporter. The truncation of PDZ binding motif in C-terminal region of UL78 did not influence the activation of NFAT.

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ABS-65341464

Development of a label-free, whole-cell assay to measure activity of the norepinephrine transporter (NET)

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Solute transport across bilayer membranes is vital for a plethora of cellular processes. In humans, the superfamily of solute carrier proteins (SLCs) constitutes the largest class of membrane-associated transport proteins. The importance of SLCs in cellular function is underlined by the involvement of these proteins in disease, making several interesting drug targets. However, in contrast to other membrane protein families – such as G protein-coupled receptors (GPCRs) – the family of SLCs has remained relatively understudied.¹

Aims

One of the hurdles faced in studying SLCs is the development of robust functional assays. Traditionally, in vitro drug screening assays often depend on the use of labeled substrates or modulators. While such assays can provide valuable information on ligand potencies, they require expensive labeling of molecules and necessitate invasive detection methods and end-point measurements. Moreover, these assays are often not suited for high-throughput screening. Therefore, there is a need for novel in vitro functional assays to study individual SLCs in a less invasive setting closer to their physiological environment.

Methods

In this project, we utilize a label-free, impedance-based technology (xCELLigence) to develop a functional assay for the human norepinephrine (NE) transporter (NET, SLC6A2) in whole cells. In this assay, changes in cell adhesion, growth and morphology are accurately monitored in real-time, without disrupting cell integrity.² Here, activation of cell surface GPCRs – in this case, adrenergic receptors – leads to changes in morphology and is used as an indirect readout of NET function. In principle, NET mediates influx of NE, thereby altering the amount of extracellular NE and thus dictating the magnitude of adrenergic receptor activation.

Results / Conclusions

Here, we demonstrate that HEK293 cells stably expressing NET are responsive to exogenous NE in the impedance-based assay. Moreover, we confirm this NE response is induced by activation of endogenous adrenergic receptors, by using receptor subtype-specific antagonists. Using known NET-selective inhibitors, we show that NET inhibition enhances the adrenergic receptor-mediated NE response. Ultimately, our assay could prove valuable in drug screening efforts for NET, with a prospect to develop similar assays for a range of SLCs.

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Discovery 2 - Receptor/Ligand and Channel/Transporter

ABS-66448306

Optical control of chemokine receptor function

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Temporal and spatial control of G Protein-Coupled Receptor (GPCR) function can be induced utilizing photoswitchable ligands. Photoswitchable ligands can reversibly photoisomerize from the trans to cis isomer upon illumination with specific wavelengths. This conformational change in the ligand may result in temporal and spatial photopharmacology. As a case study for the optical control of chemokine receptor function, we set out to develop small-molecule ligands for the chemokine receptor CXCR3 with photoswitchable efficacy in which both configurations bind the target protein but exert distinct pharmacological effects, that is, stimulate or antagonize GPCR activation. These compounds are the first GPCR azobenzene ligands with a nearly full efficacy photoswitch and may become valuable pharmacological tools for the optical control of chemokine-mediated GPCR signaling.

ABS-66058654

Characterization of cancer-related somatic mutations in adenosine A1 receptor

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Introduction

The adenosine A₁ receptor (A₁R) is highly expressed in the brain, spinal cord, and adipose tissue¹. The role of A₁R in tumor development is not well characterized yet, but A₁R activation has been reported with both anti- and pro-tumoral effects in cancer development².

A series of A₁R mutations have been found in human cancers (Van Westen, (2016). Unpublished. STW Veni project: 14410). These mutations might disturb the equilibrium of the receptor's activation states by either causing an increase (constitutively active mutants, CAMs) or a decrease in basal activity (constitutively inactive mutants, CIMs). As some residues have been found essential in receptor activation³, we wish to explore the link between receptor (in)activation and cancer-related mutations in A₁R.

Methods

In this study the yeast strain MMY24 is used, containing a chimeric G protein of which the last 5 amino acids are from a mammalian Gai3 protein⁴. This allows human GPCRs to couple to the yeast pheromone pathway and activate transcription of HIS3⁵. Thus, yeast growth is used as a read-out parameter for receptor activation.

Results/Conclusion

Dose-growth curves were obtained with a reference A₁R full agonist CPA for 13 mutant receptors (located at 7-transmembrane domain) and compared to the wild-type receptor (WT). H78L^{3,23} and S246T^{6,47} showed a 4.5-fold and 2.6-fold increase in constitutive activity, while only the high constitutive activity of S246T^{6,47} could be reduced to WT level in response to the inverse agonist DPCPX. Decreased constitutive activity and diminished

potency for CPA were observed on A52V^{2,47} and W188C^{5,46}. Moreover, A20T^{1,43}, R122Q^{4,40} and G279S^{7,44} showed decreased constitutive activity but similar potency and efficacy values for CPA in comparison to WT. Lastly, a complete loss of activation was observed in D55V^{2,50}, D55G^{2,50}, P86L^{3,31}, L134F^{4,52}, T257P^{6,58} and S267I^{7,32}.

In conclusion, the yeast system is suitable for investigating A₁R activation. Several potential CAMs, CIMs and mutants with complete loss of activation have been identified. The results obtained from this study will help in defining the role of A₁R and its mutations in tumor biology. Ultimately, this provides clues for precision medicine in cancer treatment.

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Use 1 - Regulatory affairs

ABS-66452558

Barriers and Enablers of Deprescribing Preventive Cardiovascular/ /Diabetes Medication: Health Care Professionals' Perspective

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Both the benefits and risks of preventive medication change over time for ageing patients. Not much is known about barriers and enablers for deintensifying preventive medication from the perspective of healthcare professionals (HCPs) in the Netherlands

Aims

The aim of this study was to identify barriers to and enablers of deprescribing preventive cardiovascular and diabetes medicines from the perspective of HCP and to explore the role of the pharmacist in deprescribing.

Methods

Three focus groups with in total 5 general practioners (GP), 8 pharmacists, 3 nurse assistants, 2 geriatricians, 2 elder care physicians were conducted in 3 cities in the Netherlands. Interviews were recorded and transcribed verbatim. Directed content analysis was performed on the basis of the Theoretical Domains Framework (TDF). Two researchers separately coded the data.

Results / Conclusions

Most HCPs agreed that deprescribing in this area is relevant but that barriers include poor communication between the various prescribers, insufficient reimbursement, and lack of knowledge on the subject. Some HCPs feared the deterioration of their patients' health after discontinuation their cardiovascular or diabetes medicines. All HCP stated that adequate patient communication and addressing the patients' priorities enables deprescribing. A guideline could enable the process of deprescribing such medication in elderly patients. Several HCPs stated that pharmacists could enable deprescribing by conducting medication reviews or advising about side effects and drug interactions. HCPs recognize the importance of deprescribing cardiovascular and diabetes medication as a medical decision that must be well grounded. A multidisciplinary approach including the pharmacist could support deprescribing.

ABS-66447911

Development of clinical value over the life cycle of oncology medicines in Europe: a comparison of conditional versus standard marketing authorisation

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Conditional marketing authorisation (CMA) aims to provide timely access to innovative medicines by accepting more uncertainties regarding clinical evidence at time of approval and provision of additional evidence post-approval. Until now, it is unknown if CMA medicines are of equal clinical value as standard approved medicines, and what the contribution is of post-approval evidence to determine clinical value.

Aims

To assess clinical value at different moments during the life-cycle (market entry, conversion to standard approval, end-of-follow-up) for conditional versus standard approved oncology medicines. In addition, to determine the contribution of data requested by regulators in the context of CMA to clinical value estimates.

Methods

We identified all oncology medicines conditionally approved by the European Medicines Agency (conditionals) that were converted to standard approval between July 2006 and February 2019. All conditionals were matched with one or more standard approved oncology medicines (controls), considering similarity in indication. We will longitudinally assess their clinical value per indication based on relevant studies which can be found in the European Public Assessment Reports, for e.g. extensions of the indication, and those submitted specifically for conditionals by making use of the European Society for Medical Oncology Magnitude of Clinical Benefit Scale (ESMO-MCBS). Ultimately, we will compare the clinical value of the conditionals at time of conversion with that of the controls at time of standard approval and at end-of-follow-up.

Results

In total 14 conditionals and 22 controls were identified, that underwent at median 2 vs. 1 extensions of the indication respectively. An initial detailed search of the regulatory domain identified 134 vs. 280 studies (median 8.5 vs. 9) that based on their endpoints seem applicable for the ESMO-MCBS. In the next step, the ESMO-MCBS clinical values will be determined.

Conclusions

More extensions of indication were found for the conditionals. One reason might be that these medicines enter the market with more uncertainties regarding clinical evidence, generally leading to smaller indications. Of note, this study is ongoing, data on clinical value will be available shortly.

ABS-66122325

Medicinal products approved with Quality by design (QbD) principles incorporated into their development in the EU between 2014 and 2018

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Quality by design (QbD) is a systematic risk-based approach to development with predefined characteristics and a quality risk management throughout the life cycle of a product. QbD aims to ensure the desired quality of the product by assessing the variables which might impact the quality. ICH Q8-Q11 give guidance for QbD applications, in particular ICH Q8 (R2) approved in 2009 describes the principles of QbD.



Use 1 - Regulatory affairs

Aims

- To evaluate the number of medicinal products approved with QbD principles incorporated into their development;
- To analyse the type of products and the formulations developed using a QbD approach.

Methods

The EMA website was checked for the medicinal products approved between 2014 and 2018. The EPARs of these products were searched for "quality by design or QbD". All the obtained QbD applications were further classified based on the key words in the EPAR as below:

Type of QbD application	Key words in the EPAR
Full	QbD principles/approach/tools/strategy/paradigm/methodology
Partial	contains QbD elements/a combination of conventional and QbD elements
Mixed	a combination of full and partial QbD for AS and DP
No QbD	QbD not mentioned in the relevant active substance (AS)/drug product (DP) sections

Furthermore, information on the type of products (small molecules/biotech-derived/generics) and pharmaceutical forms (tablets/solutions) was collected.

Results/Conclusion

Out of 432 medicinal products approved, 99 products were manufactured with QbD during the development (23%). These 99 products were further classified as below:

Type of QbD application	Number of products approved
Full	49
Partial	44
Mixed	6

Majority of the products belong to 'small molecules' category (84%); remaining are biotech-derived medicinal products (16%) and generics (6%). In the period studied there was an increase the number of biotech-derived products and generics using QbD in their development. A wide variety of formulations was found within small molecules, while all the biotech-products are available as solutions for injection.

Although QbD is not mandatory, it was expected to be applied frequently. However, it's not the case: in 77% of the products approved, there was no QbD. The present study showed that only 55 (49 + 6) out of 99 medicinal products were developed using full QbD between 2014 and 2018. Among these, 58% were developed by nine pharmaceutical companies.

ABS-66314207

IVD assays and EMA authorised anticancer medicinal products in the context of EU IVD Regulation

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Introduction

Predictive biomarker (BM)-based assays are critical tools for implementation of precision medicine in a clinical setting.

As defined in the new EU Regulation 2017/746 on In-Vitro Diagnostic Devices (IVDR), in some cases, an IVD assay measuring a BM will be considered a companion diagnostic (CDx) when utilised in support of safe and effective use of a medicinal product.

Based on this definition one could argue that for an IVD to be classified as CDx relevant information should be included in relevant sections of Summary of Product Characteristics (SmPC).

Aims

To assess IVD assay information included in SmPCs of anticancer medicinal products approved by European Medicines Agency (EMA) in the context of IVDR.

Methods

SmPCs of anticancer medicinal products authorised by EMA between 2010 and 2018 were assessed to identify and classify products that included in 'Therapeutic indication (SmPC section 4.1)' a predictive BM.

Results/Conclusions

Between 2010 and 2018 there were 82 anticancer medicinal products authorised by EMA. Predictive BMs were included in SmPC section 4.1 of 35/82 products.

For 21/35 products the use of a BM assay was described in 'Posology and method of administration (SmPC section 4.2)' and/or 'Special warnings and precautions for use (SmPC section 4.4)'. In the context of IVDR such products would be considered a CDx as they are 'essential for the safe and effective use of a corresponding medicinal product'. In all cases, the use of a 'validated' assay was specified.

For 3/35 products a recommendation was included in SmPC section 4.4 aiding in benefit-risk decision making for therapeutic use. Such wording is more in-line with FDA's definition of complementary diagnostics which is not encompassed by IVDR. For 11/35 products, although a BM was included in the indication, no information regarding the use of a BM assays was included in SmPC sections 4.2 or 4.4. BMs in question were only mentioned in 'Pharmacodynamic properties (SmPC section 5.1)' in the context of clinical efficacy and safety studies conducted.

For almost 50% of anticancer medicinal products for the period studied BM information was included in 'Therapeutic indication (SmPC section 4.1)'. Most of the products could be classified as CDx, but for some this was not possible (e.g. 'complementary diagnostics').

ABS-66122439

Approval of anticancer medicinal products in the EU requiring no toxicology studies

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ICH S6 and S9 provide specific guidance on the requirements for anticancer medicinal products and biotech-derived medicinal products. In these guidelines, it is argued that less toxicology studies could be required for these medicinal products, and the need for toxicology studies is often decided on a case-by-case basis and/or the availability of a suitable animal model. This could result in a situation that for some products only limited toxicology studies to even no toxicology studies are conducted.

Aim

To evaluate if there are anticancer medicinal products approved for which no toxicology studies were required as suggested by the guidelines.

Methods

The EMA website was searched for anticancer medicinal products approved between 2010 and 2018. Drugs with no toxicology studies were selected for analysis. For a certain class of medicinal products, toxicology studies may not be required according to the guidelines, but for particular reasons, the applicant might have decided to conduct one toxicology study. Therefore, medicinal products for which one toxicology study was conducted were also selected for analysis. The European public assessment reports (EPARs) of these medicinal products were checked for relevant justification for omitting toxicology studies.

Results/Conclusions

Overall 82 anticancer medicinal products were selected in the period 2010-2018. From these, three products were identified

Use 1 - Regulatory affairs

according to the selection criteria. Two advanced therapy medicinal products (ATMPs) (tisagenlecleucel and axicabtagene ciloleucel) were selected for which no toxicology studies were conducted. This has been justified due to the type of product (patient-specific) and due to the lack of representative in vitro assays, ex vivo or in vivo models which can accurately address the toxicological characteristics of the human product.

One biotech-derived medicinal product (elotuzumab) was selected for which only a single dose toxicity study in rhesus monkeys was conducted. This has been justified due to the lack of species-specific cross-reactivity and absence of relevant animal species or transgenic mouse models. This is also due to the binding properties of elotuzumab to the target antigen, which is strictly human specific and the non-feasibility of expressing this human-specific antigen in T-cells.

Overall in the last nine years, three medicinal products were found for which no toxicology studies were required.

ABS-66227767

Stakeholder Perspectives on Implementing Precision Medicine in Diabetic Kidney Disease

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Aims

One of the important aims of the Innovative Medicine Initiative BEAt-DKD consortium is to promote implementation of Precision Medicine (PM) in treating diabetic kidney disease (DKD). Engaging stakeholders is crucial in this process. We held a consensus workshop and conducted a survey of diabetes stakeholders to identify benefits and obstacles of PM, and to strategize solutions.

Methods

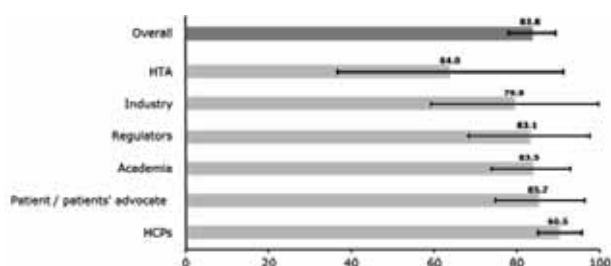
Seventy-one participants from 26 countries met in Amsterdam, the Netherlands over 2 days to develop a strategy to move PM forward in DKD. Represented stakeholder groups included patients with diabetes and advocates (n=11), academia (n=19), drug regulators (n=7), health technology assessors (HTAs)(n=6), industry (n=10), and health care providers (HCPs)(n=18). A survey was developed and pilot tested prior to implementation. Respondents were asked about their opinions on need, benefits and obstacles for introducing PM in DKD. A consensus discussion was held to strategize solutions.

Results/Conclusions

Stakeholders were mostly positive for PM in DKD (Figure 1). HTAs least agreed, while HCPs most agreed. Obstacles and concerns for PM included data safety, time constraints, and increased burden for assessments. Keys to successful implementation of PM would be increased engagement with patients, specific training for HCPs in PM, and early collaboration between stakeholders. All stakeholders responded that quality of life outcomes would be important to assess the impact of PM.

In conclusion, diabetes stakeholders view PM in DKD positively. Implementing PM is complex as different stakeholders have different priorities. The consensus of all stakeholders was that

early engagement and aligning stakeholders goals are critical to implement PM in DKD.



ABS-66449550

The Junior-Adverse Drug Event Managers

Spontaneous reporting of adverse drug reactions (ADRs) is a cornerstone in pharmacovigilance which is highly dependent on the quantity and quality of reported ADRs. Most interventions to improve this problem in healthcare professionals have been unsuccessful. Adverse drug event managers have been successfully implemented in numerous hospitals in Denmark where they have shown to ease the pressure for clinicians and increase the number of Individual Case Safety Reports (ICSR). Since screening and reporting of these ICSR are of great value for educating our future doctors on pharmacovigilance we started a junior-adverse drug event managers team (J-ADEMs). This study aims to investigate the feasibility of a J-ADEMs team and evaluate the clinical and educational value of this innovative intervention.

The J-ADEMs program was set up as a prospective longitudinal intervention study to detect and report possible ADRs on and during hospital admission. The J-ADEM team, consists of medical students (1st – 6th year), functioning as a passive and active service to manage and report medication related side effects. The J-ADEMs can be contacted by phone or email but they also actively screen the internal medicine ward for ADRs. When an ADR is detected the team report the ADR to the Netherlands pharmacovigilance centre Lareb, answer all follow-up questions and update the electronic patient record. After the report, all patients evaluate the J-ADEM team and physicians were asked why they had not reported the serious ADR themselves.

From October 2017 to January 2018, 35 patients with 116 problem list symptoms were analyzed by the J-ADEMs team. Most patients (27) were actively screened by the J-ADEMs team while eight signals of possible ADRs were received by healthcare professionals. Of the 116 problem list symptoms, 25 ADRs were reported to the pharmacovigilance centre of which 20 were classified as serious ADRs. Compared to 2016 this showed a 300% increase in the number of ICSR by managing only one (of +/-20) ward. Most reports were on electrolyte disorders (n=11) or haematological disorders/bleeds (n=6) and were related to diuretics (6) and acetylsalicylic acid (4). Patient satisfaction was 7.9 (1-10, min-max) and 87% of patients found it (extremely) relevant that a ICSR was made when the ADR caused the hospital admission. All physicians agreed the symptoms were at least related to the medicine, however most frequently reported: indifference (14) and ignorance (7) as reasons they didn't report the ADR themselves.

The J-ADEMs team is an highly innovative healthcare improvement for hospitals. Not only does this team fastly increase the number of ICSR, it also has the opportunity to increase pharmacovigilance awareness in current and future healthcare professionals. Further plans are to expand the service to other wards, evaluate the educational value for students and analyze the quality of the students ICSR.



Use 2 - Pharmacokinetics/dynamics and Adverse drug ...

ABS-66436907

Association between drug-gene-interaction, drug-drug-interaction, and drug-drug-gene-interaction and early discontinuation, switching and dose adjustment of (es)citalopram: the PharmLines Initiative.

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(Es)citalopram users commonly fail to achieve symptom remission in their first treatment episode. Drug-gene-interaction (DGI), drug-drug-interaction (DDI), and drug-drug-gene-interaction (DDGI) may influence the efficacy of (es)citalopram.

Aims

We aimed to explore the association between DGI, DDI, and DDGI and the effects on (es)citalopram dispensing course.

Methods

An inception cohort study was conducted as part of the PharmLines Initiative among adult Caucasians (≥ 18 years old) from the Lifelines cohort (167,729 participants) with linked dispensing data from the University of Groningen prescription database IADB.nl and available genetic information on CYP2C19/3A4 genotypes. Exposure groups were first-time users of (es)citalopram with (1) DGI (CYP2C19/3A4 deviating phenotype), (2) DDI (CYP2C19/3A4/2D6 inhibitors/inducers), and (3) DDGI (co-presence of DDI and DGI). Outcomes were drug switching or dose adjustment, and early discontinuation within 90 days after the start of (es)citalopram. We applied logistic regression modeling to estimate adjusted odd ratios with corresponding 95% confidence interval.

Results

Overall, 316 (es)citalopram starters (median 45 years, 62% women) with complete genetic information were identified. DGI between (es)citalopram and decreased/no function of CYP2C19 tended to increase the odds of switching and dose reduction, but reduced the risk of discontinuation regardless of CYP3A4 phenotypes. The associations were probably dominated by the CYP2C19 intermediate metabolizer genotype which was associated with more switching [OR: 2.76; 95% CI: 1.15-6.62], dose reduction [OR: 4.16; 95% CI: 0.78-22.27] and less early discontinuation [OR: 0.35; 95% CI: 0.15-0.79]. Meanwhile, DDI and DDGI showed a trend towards increasing the odds of dose reduction [OR: 4.87; 95% CI: 0.29-82.07] and switching [OR: 2.61; 95% CI: 0.46-14.78], respectively.

Conclusions

DGI involving decreased function of CYP2C19, regardless of CYP3A4 phenotypic status, may increase the risk of switching or dose reduction of (es)citalopram. For DDI and DDGI, trends towards dose reduction and switching, respectively, were observed, but larger studies are needed to confirm these findings.

ABS-66444410

Hypersensitivity reactions by excipients in patches: a review of literature and spontaneous reports in the Netherlands

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Aims

Transdermal patches may cause hypersensitivity reactions by the pharmacological active ingredients or the excipients, and mechanical reactions by application or removing the patch. [1,2] In this study we will review the excipients that may cause hypersensitivity reactions and analyse spontaneous reports for hypersensitivity reactions associated with patches in the Netherlands.

Methods

A systematic Pubmed Search was performed that identified publications with hypersensitivity reactions to transdermal patches. All spontaneous reports with patches reported to the Netherlands Pharmacovigilance Center Lareb (1991-2018) were analysed with respect to the pharmacological active agent, and the type of reaction.

Results / Conclusions

In total, 304 hypersensitivity reactions were reported to Lareb. The most frequently reported reactions of transdermal patches were local skin reactions with edema or pain. Most reports concerned buprenorphine and rivastigmine patches. In the spontaneous reports, it was seldomly documented if the hypersensitivity reactions were caused by the pharmacological active ingredient or by one of the excipients. The review of literature showed, that hypersensitivity reactions occur less frequent, whereof allergic contact dermatitis (type IV-allergic reaction) is most frequently reported. Allergic contact dermatitis can be caused by both the active substance or by excipients in the transdermal patch. Excipients such as adhesives, anti-oxidants, and excipients stimulating skin-hydration or penetration of the active ingredient, are all associated with hypersensitivity reactions, mostly contact dermatitis. Type I-allergic reactions, such as urticaria, bronchospasm and angioedema, are rare and usually caused by the active substance. For type IV-allergic reactions requiring an alternative treatment and for type I-allergic reactions, it is advisable to perform allergy testing to determine the allergen.

Documenting hypersensitivity reactions in the electronic health system is important to prevent a hypersensitivity reaction in the future. Excipients can be documented as causative agent for hypersensitivity reactions.

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Use 2 - Pharmacokinetics/dynamics and Adverse drug ...

ABS-66449053

Handling information about new drug safety issues in Dutch hospitals

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Introduction

To optimise communication about new drug safety issues it is important to align with current systems and routines for handling drug information in medical practice. Previous research on optimising risk communication in the Netherlands was mainly focussed on individual healthcare professionals (HCPs). Many new safety issues communicated by regulators through Direct Healthcare Professional Communications (DHPC) are directed at HCPs that work in a hospital setting. Little is known about the systems and routines that hospitals have to handle such information.

Objectives

Which systems and routines for handling information about new drug safety issues exist in Dutch hospitals?

Methods

Using a topic list based on the Theoretical Domain Framework, semi-structured focus group interviews are being conducted with hospital-based specialists and pharmacists.

Results

In this ongoing study 15 hospitals have been approached, of which seven agreed to participate. Focus groups have already been conducted in 6 hospitals three academic, two top clinical and one general hospital. Specialists who participated in the groups included gynaecologists, cardiologists, neurologists, internists, paediatricians, rheumatologists, haematologists, and geriatricians. All hospitals had committees concerning antibiotic policy. Most hospitals had a drug formulary committee that reviews new medicines; only some of these committees reviewed drug safety issues. In one hospital DHPCs were discussed in the drug formulary committee, while in another hospital the DHPCs were placed on intranet. In yet another hospital it was mentioned that new drug safety issues are being addressed by individuals at department meetings. In some hospitals pharmacists were involved in drafting or updating treatment protocols, and DHPCs could be a reason to alter these treatment protocols or the electronic prescribing system (EPS). Opinions regarding the EPS differed per participant, however, overall most believed that EPS could play a role to incorporate new safety information.

Conclusion

Most hospitals did not have clear systems or routines in place to handle DHPCs even though DHPCs could lead to updates of treatment protocols and EPS.

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Sex differences in reported adverse drug reactions are primarily seen in the first weeks after metformin initiation

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Introduction

Previously it has been shown that women more often experience a metformin-associated adverse drug reaction (ADR) than men¹. Information on when this difference occurs over time after metformin initiation and its concurrence with the prescribed dose may shed light on the involved mechanisms on sex differences.

Aims

The aims of this study were to assess whether sex differences in reported ADRs for metformin are observed at different time periods after initiation, and to explore their concurrence with sex differences in the dose of metformin over time.

Methods

This study had a longitudinal design using data about patients initiating metformin collected by the Netherlands pharmacovigilance center Lareb through their Intensive Monitoring program. Patients were asked to complete a web-based questionnaire at six time periods after initiation (i.e. 2, 6 weeks, 3, 6, 9, 12 months). The outcome variables were the proportion of patients reporting any ADR (primary) and the dose of metformin (secondary). Sex differences in the proportions of ADRs and in the dose were tested at each time period using respectively Pearson Chi-Squared tests and Wilcoxon rank-sum tests. Using Bonferroni adjustment for multiple testing, a P-value <0.01 was considered statistically significant.

Results

The number of included patients was 1,712 (40.9% women). Women reported more often an ADR than men, which was statistically significant at the assessment at 2 weeks (34% versus 25%, P<0.001), and 6 weeks (37% versus 28%, P=0.001) after initiation. In general, women reported to be prescribed a lower dose than men which became statistically significant at the 9 months assessment (P<0.01).

Conclusions

Sex differences in the reporting of ADRs were seen in the first weeks after metformin initiation, whereas differences in dosing were observed after several months. Patients, in particular women, might benefit from being prescribed lower metformin doses at treatment initiation.

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Effects of Dapagliflozin on Volume Status when added to Renin-Angiotensin-System inhibitors

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Sodium glucose co-transporter 2 (SGLT2) inhibitors reduce the risk for heart failure events in patients with type 2 diabetes at high cardiovascular risk. These effects are thought to be attributed at least in part to diuretic effects. Previous non-placebo controlled studies with SGLT2 inhibitors observed changes in volume markers in healthy individuals and in patients with type 2 diabetes with preserved kidney function. It is unclear whether patients with type 2 diabetes and signs of kidney damage show similar changes.

Aims

The aim of this study was to assess the effects of SGLT2 inhibitors dapagliflozin on volume markers in patients with type 2 diabetes and elevated albuminuria.

Methods

A post-hoc analysis was performed of two randomized placebo controlled cross-over trials, the IMPROVE study (N=33) and the DapKid study (N=36), assessing effects of dapagliflozin 10 mg/day when added to renin-angiotensin-system inhibition in patients with type 2 diabetes and urinary albumin-to-creatinine ratio ≥ 30 mg/g. Blood and 24-hour urine was collected at start and the end of treatment periods lasting 6 and 12 weeks. Effects of dapagliflozin compared to placebo on various markers of volume status were determined.

Results / Conclusions

Dapagliflozin increased urinary glucose excretion by 217.2 mmol/24h (95% CI: 155.7 to 278.7, $p<0.01$) and urinary osmolality by 60.4 mOsmol/kg (30.0 to 90.9, $p<0.01$), compared to placebo. Fractional lithium excretion increased by 19.6% (6.7 to 34.2, $p<0.01$), suggesting inhibition of sodium reabsorption in the proximal tubule. Systolic blood pressure and body weight decreased after 6 to 12 weeks treatment by 5.7 mmHg and 1.3 kg. Renin and copeptin increased by 46.9% (21.6 to 77.4, $p<0.01$) and 33.0% (23.9 to 42.7, $p<0.01$). FWC was reduced by -885.3 ml/24h (-1156.2 to -614.3, $p<0.01$ vs placebo) likely as a result of increased copeptin levels.

These changes in markers of volume status suggest that dapagliflozin exerts both osmotic and natriuretic diuretic effects in patients with type 2 diabetes and kidney damage, as reflected by increased urinary osmolality and fractional lithium excretion. As a result, compensating mechanisms are activated to retain sodium and water.

ABS-64783122

High dabigatran concentrations in elderly patients

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Introduction

Many geriatric patients have an indication for oral anticoagulants, like dabigatran.[1] Efficacy of dabigatran is preserved above 75 years, but bleeding risk increases.[2] Routinely measuring plasma concentrations of dabigatran is not advised, although inter-patient variation is known to be high, and an association is seen between concentration and bleeding risk. This risk rises if plasma concentrations are above 150 ng/ml.[3]

Objectives

Aim of this study was to explore variation in dabigatran concentrations in patients aged above 75 years.

Methods

We calculated the proportion of elderly patients with trough levels above 150 ng/ml, and searched for clinical parameters associated with high trough levels in this population. 50 Patients were recruited from cardiology and geriatric departments of a large hospital in the Netherlands. They received dabigatran because of atrial fibrillation. Exclusion criteria were use of other medication with pharmacokinetic or pharmacodynamic interactions with dabigatran, estimated glomerular filtration rate (eGFR) below 30 ml/min, transaminase elevation, actual malignancy, or being incapacitated.

Results

We found a dabigatran trough concentration above 150 ng/ml in 33%. In patients with impaired renal function (30-60 ml/min) or BMI < 20 kg/m², the incidence of elevated concentrations was two times higher.

Conclusion

Elderly patients with eGFR between 30 and 60 ml/min or low body mass index are at increased risk for elevated trough concentrations of dabigatran, which can cause additional bleeding risks.

Further research should be undertaken to find out whether dose adjustment is advisable for elderly patients with these risk factors.

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ABS-66443402

Pharmacokinetics and target attainment of antibiotics in critically ill children – a systematic review of current literature

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Introduction

Pharmacokinetics (PK) are severely altered in critically ill patients due to changes in volume of distribution (Vd) and/or drug clearance (Cl). To what extent this affects the PK of antibiotics in critically ill children is largely unknown.

Objectives

To identify gaps in current knowledge and to compare published PK parameters of antibiotics in critically ill children to healthy children and critically ill adults.

Methods

Systematic literature search in PubMed, EMBASE and Web of Science. Articles were labelled as relevant when they included information on PK of antibiotics in critically ill, non-neonatal, pediatric patients. Extracted PK-parameters included Vd, Cl,

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trough concentrations, AUC, probability of target attainment, and elimination half-life.

Results

45 relevant articles were identified. Studies focusing on vancomycin were most prevalent (15/45). Other studies included data on penicillins, cephalosporins, carbapenems and aminoglycosides, but data on ceftriaxone, ceftazidime, penicillin and metronidazole could not be found. Critically ill children generally show a larger Vd and higher CI than healthy children and critically ill adults. Reduced target-attainment was described in critically ill children for multiple antibiotics, including amoxicillin, piperacillin, cefotaxime, vancomycin, gentamicin, teicoplanin, amikacin and daptomycin. 32/45 articles included information on both Vd and CI, but a dosing advice was given in only 18 articles.

Conclusion

The majority of studies focus on agents where TDM is applied, while other antibiotics lack data altogether. The larger Vd and higher CI in critically ill children might warrant a higher dose or extended infusions of antibiotics in this patient population to increase target-attainment. Studies frequently fail to provide a dosing advice for this patient population, even if the necessary information is available. Our study shows gaps in current knowledge and encourages future researchers to provide dosing advice for special populations whenever possible.

ABS-66443479

External validation of model-based dosing guidelines for vancomycin, gentamicin and tobramycin in critically ill children

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Introduction

Pharmacokinetic models are frequently used to simulate dosing strategies for special populations, including critically ill children. The Dutch Pediatric Formulary (DPF) partially bases its guidelines on these models. However, prospective validation of updated dosing regimens is rare.

Objectives

To identify target attainment and safety of vancomycin, gentamicin and tobramycin after a dose update in the DPF for critically ill neonates and children.

Methods

Retrospective cohort study in PICU and NICU patients receiving vancomycin, gentamicin or tobramycin between January 2015 and March 2017 in 2 university hospitals. Demographic clinical laboratory and TDM-data were collected. Target (steady state) trough concentrations for vancomycin, gentamicin and tobramycin used were 10-15, ≤ 1 and ≤ 1 mg/l, respectively. Target gentamicin peak concentrations used were 8-12 mg/l.

Results

486 patients were included in total (165 vancomycin, 97 gentamicin and 224 tobramycin). Trough concentrations of vancomycin, gentamicin and tobramycin were within the target range in 37.5%, 85.3% and 77.2% of patients, respectively. Target attainment of gentamicin peak concentrations in NICU patients was 31%. Non-target trough concentrations were most

prevalent in term NICU patients (vancomycin 70%, gentamicin 26% and tobramycin 36.8%). Gentamicin peak concentrations were subtherapeutic in 91% and 45.5% for term and preterm NICU patients, respectively. Creatinine concentrations correlated positively with antibiotic concentrations (correlation coefficient range 0.46-0.54, $p \leq 0.01$ in all cohorts).

Conclusion

Despite recent model-based dosing alterations, sub- and supratherapeutic concentrations of vancomycin, gentamicin and tobramycin are still frequent in critically ill children. Linear dose alterations did offer improvements in target attainment, but did not fully address all relevant covariates that contribute to the large interindividual variation in clearance and/or volume of distribution in these patients. Creatinine clearance was consistently correlated with concentrations of all 3 drugs, but future research is needed to identify whether including this parameter in dosing can improve target attainment and safety.

ABS-66438239

A pharmacokinetic evaluation of oral Clavulanic Acid in term newborns

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Background

Clavulanic acid is an irreversible beta-lactamase inhibitor. It has a weak antibacterial action on its own, but when combined with a beta-lactam antibiotic such as amoxicillin, it is effective against a broad range of bacteria. Despite its widespread use, little is known on the mechanism of action and target levels. A few studies on oral clavulanic acid in adults are available reporting great variance (AUC median 4.99mg·h/L [0.44-8.31]) (1) and a short elimination half-time (1.08h). (2) Observations in neonates are currently lacking. We therefore evaluated the pharmacokinetics of oral clavulanic acid co-administered with amoxicillin in term newborns.

Methods

As part of a multicenter RCT (Clinicaltrials.gov:NCT03247920) evaluating neonatal intravenous-to-oral switch therapy in probable bacterial infection, we measured serum levels in patients allocated to the intervention group. They switched to amoxicillin/clavulanic acid suspension (25/6.25mg/kg tid), after 48 hours of intravenous penicillin/gentamicin. Two blood samples from different dosing intervals, were obtained and directly stored at -80°C. Initially, and to ensure that amoxicillin



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levels were attained as safety marker, levels in the second part of the timeframe (4-8h after administration) were collected. For the second batch, peak levels (1-2h after administration) were collected. Analysis was performed using Liquid Chromatography and Mass Spectrometry.

Results

Samples of 30 patients were analyzed. Through levels (n=44) were collected 6.0±1.3h after antibiotic administration. Top levels (n=13) were collected on average 1.6±0.5h after administration (mean±S.D.). Clavulanic acid levels were detected in all patients but a great variance was observed. Trough level: 1.4mg/L (0.20-4.82); top level: 1.9 mg/L (0.39-6.79); median (range). AUC's in individual patients (n=3) were in range with reported AUC's in literature.

Conclusions

Oral clavulanic acid is absorbed in term newborns, but a great variance is seen. AUC's following oral administration are comparable to those of children and adults.

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ABS-66446786

Quantifying Midazolam Pharmacodynamics in Critically-Ill Mechanically Ventilated Children Using a Parametric Time to Event Analysis

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Objectives

While knowledge on the pharmacokinetics (PK) of midazolam in children of various ages and disease conditions is increasing, there is only limited information on the pharmacokinetic-pharmacodynamic (PK-PD) relationship of midazolam in critically-ill children. Analyzing clinically-relevant, ambiguous endpoints in the absence of objective methods of quantitation, such as pain and sedation, remain of interest and often require novel PD modelling approaches.

Aims

In this study, we aimed to investigate the effect of midazolam on sedation in critically-ill, mechanically ventilated P-ICU patients using a parametric time to event (PTTE) analysis.

Methods

Data was derived from a multi-institutional randomized clinical trial (<http://www.trialregister.nl/trialreg/index.asp>, no. NTR2030) aiming at assessing the efficacy of Daily Sedation Interruption (DSI) in critically-ill, mechanically ventilated P-ICU patients, by replacing midazolam with blinded infusions of either placebo or midazolam. Sedation was monitored at two-hour

intervals until patient discomfort required the re-commencement of midazolam. The time to re-commencement was the main outcome in the current analysis. Patient PK information was available from a population-PK (pop-PK) model developed on a subset of 83 patients from this study.²

Modelling was used to characterize the probability of requiring midazolam re-commencement in NONMEM 7.3. Individual predicted midazolam plasma concentration (IPRED) at the time of reinfusion, as well as midazolam exposure (calculated as AUC) until re-commencement, were tested as covariates, along with patient age (days), sex, organ failure and inflammation.

Results

A total of 138 sedation-interruption observations were made in 79 patients, who were also part of the PK analysis. A constant baseline hazard best described the data. Patients receiving a blinded placebo in the trial had a 56% higher hazard to require re-commencement of midazolam compared to patients receiving blinded midazolam.

Conclusions

The results of using parametric time to event demonstrate the sedative effect of midazolam in mechanically-ventilated P-ICU patients. Further model development and evaluation of covariates is warranted to get to predictive models for dosing midazolam in P-ICU children.

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Comparison of myelotoxicity and nephrotoxicity between daily low-dose cisplatin with concurrent radiation and cyclic high-dose cisplatin in non-small cell lung cancer patients

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Potential antineoplastic effect of cisplatin, the first line of treatment in non-small cell lung cancer (NSCLC), is hindered by its nephrotoxicity and myelotoxicity. Two different cisplatin dosing regimens are currently used in the treatment of NSCLC: a cyclic high-dose and a daily low-dose regimen.

Aims

The aim of this study is to assess the risk on myelotoxicity and nephrotoxicity from the daily low-dose cisplatin (DLD) treatment as compared to cyclic high-dose cisplatin (CHD).

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Methods

A retrospective cohort study was conducted by including NSCLC patients from the Amsterdam UMC and Antoni van Leeuwenhoek cancer hospital, treated with cisplatin between 2011 and 2018. Myelotoxicity and nephrotoxicity were defined based on common terminology criteria (CTCAE v4.03) and categorized as \geq grade 1 and \geq grade 2. Adjusted relative risks (adjRR) were calculated with modified Poisson regression and adjusted hazard ratios (adjHR) were calculated with Cox regression models.

Results / Conclusions

Of the 115 NSCLC patients receiving DLD (N=62) and CHD (N=53), 60% had \geq grade 1 anemia, 33.9% leukopenia, 31.3% neutropenia, 27.8% thrombocytopenia, 32.2% acute nephrotoxicity and 58.3% chronic nephrotoxicity. In the DLD group less \geq grade 2 toxicities were reported compared to the CHD group except for acute nephrotoxicity. However, there was a stronger association in the DLD group with \geq grade 1 leukopenia, thrombocytopenia, and nephrotoxicity. The DLD group developed significantly more \geq grade 1 leukopenia (adjRR=1.83, 95%CI 1.02-3.27), thrombocytopenia (adjRR=3.43, 95%CI 1.64-7.15) and \geq grade 2 acute nephrotoxicity (adjRR=3.02, 95%CI 1.20-7.56). The DLD group had a lower adjusted cumulative hazard for developing \geq grade 2 myelotoxicity and chronic nephrotoxicity but not for acute nephrotoxicity (adjHR=3.90, 95%CI 1.35-11.23). Overall, the DLD regimen was safer than the CHD regimen when assessing the risk of \geq grade 2 myelotoxicity and chronic nephrotoxicity. However, this might not be the case in patients with a higher risk of acute nephrotoxicity.

ABS-66449407

Stimulating and facilitating European collaboration in clinical pharmacology and therapeutics education

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Introduction

European medical students are insufficiently trained to become safe and effective prescribers and there are large differences in the way clinical pharmacology and therapeutics (CPT) is taught across European countries¹. Digital educational resources were recently shown to be effective in teaching knowledge and skills required for rational prescribing and sharing and collaboratively creating such resources across Europe was proposed as means to improve and harmonize CPT education². Therefore, we aim to facilitate and stimulate international collaboration and sharing of resources in the field of CPT education via an online platform.

Aims

The aim of this study is to assess the attitude and readiness of European CPT educators towards sharing existing educational resources and creating new ones and identify the potential barriers towards doing so. We aim to establish the framework for online sharing platform.

Methods

An online survey was sent to the principal CPT educators of 281 medical schools in Europe. Open-ended questions were analyzed using a thematic analysis.

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Results / Conclusions

The principal educator from 95 (34%) EU medical schools responded. 70% of EU medical schools make use of one or more resources in their curriculum, the median number of resources is 2 (varying from 1 to more than 20). Only 30 (45%) of medical schools currently collaborate in the field of digital education. 99% of educators say they feel that sharing resources is beneficial. Perceived reasons to use the platform include: greater quality resources (cited by 26 participants), more access to resources (25), harmonization (18) and collaboration (14). The barriers were mostly: Language barriers (29), local differences in guidelines (21) and the required investment of time and money (21).

Conclusion

The EU CPT educators feel positively about an online collaboration platform for sharing digital resources. The facilitators and barriers were discussed in an international consensus meeting during EACPT Stockholm 2019 and will be used as framework for the platform.

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Clinical decision support application for gastric ulcer prophylaxis versus usual care: a case-based self-controlled trial

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Introduction

Unintentional guideline deviations are problematic. Clinical decision support systems (CDSS) have been shown to aid guideline adherence rates. However, CDSS are currently not universally available, costly to produce and hindered by alert fatigue. To reduce these problems, we have created the DrugChoice platform that can turn any guideline into a clinical decision app that is available on-demand on mobile and desktop devices. Because up to 57% of protonpump inhibitors (PPI) is not indicated and the national guideline for gastric ulcer prophylaxis (NHG) is complex, we have translated this guideline in a DrugChoice application.

Aims

To evaluate the guideline adherence with use of the application as compared to usual care.

Methods

Current (junior doctors) and future (medical students and nurse-prescribing trainees) prescribers were asked to solve two sets of 5 cases in a self-controlled trial. Case-sets were randomized, after which the first set was solved with care as usual (clinical expertise or guideline) and the second with the application. Participants also answered a short survey about user friendliness.

Results

20 medical students (MS), 30 nurse-prescribing trainees (NPT) and 11 junior doctors (JD) rated themselves 2.9 ± 0.6 knowledgeable about the NHG guideline on a 5-point Likert



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scale. The number of correct PPI prescriptions with usual care ranged from 51% for NPT and 52% for MS to 65% for JD. Using the application the percentage correct increased to 78% for NPT ($P < .001$), to 83% for MS ($P < .001$) and 89% for JD ($P = .03$). The 50 participants (5JD) that performed usual care with the guideline performed better on the usual care part than the 11 participants (6JD) that based answers on their clinical expertise (67% vs 51%) but they performed worse with application (72% vs 84%). More than 80% agreed or completely agreed that they will use the app in real practice, they trust the advices and find the applications user-friendly.

Conclusion

The app is more effective than guideline or usual care for gastric ulcer prophylaxis indications.

ABS-66447151

Drug-drug interactions with risk for serotonin toxicity: practice recommendations based on a systematic evaluation of pharmacological evidence

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Introduction

Serotonin toxicity (ST), also called serotonin syndrome, is a cluster of drug-induced, dose-related adverse effects that are caused by increased serotonin levels in the central nervous system. [1] The presentation of ST is variable, ranging from mild to life-threatening symptoms, and many case reports, expert opinions and reviews have been published about drugs associated with ST.

Aim

To support professionals in rational use of (combinations of) serotonergic drugs, and to give meaningful suggestions for actions, we aimed to develop practice recommendations for drug-drug interactions associated with serotonin toxicity.

Methods

Drugs associated with serotonergic toxicity were identified in medical handbooks, in reviews, and by an analysis of cases reported in a meta-analysis.[2] The drugs associated with ST were evaluated with respect to their proposed pharmacologic mechanism of influencing synaptic serotonin levels, the evidence supporting a relation between the drugs and serotonergic activity, and the clinical relevance to include drugs in a potential alert for a drug-drug interaction in the context of clinical decision support. The evaluation was performed by a multi-disciplinary expert panel.

Results

Three classes of drugs were considered to be related with serotonergic toxicity based on their pharmacologic mechanism: drug stimulating serotonin release, mono-amine oxidase inhibitors, and drug inhibiting serotonin reuptake. Major classes of drug in these groups are amphetamines/ecstasy, Monoamine oxidase (MAO)-inhibitors and specific serotonin reuptake inhibitors (SSRIs). Some drug that has been associated with serotonergic toxicity, was not considered relevant in the context of clinical decision support. Among these were mirtazapine and triptans. Severe reactions are merely caused by a combination

of two or more serotonergic drugs that influence serotonin by different pharmacological mechanisms.[3] Hence these combinations should not be used simultaneously because of increased risk for ST. Drugs influencing serotonin by the same mechanism can be combined if needed.

Conclusion

Combinations of MAO-inhibitors, drug inhibiting serotonin reuptake and drug stimulating serotonin release should be avoided. Therefore, these combinations should be alerted by a clinical decision support system. Inclusion of drug in such alerts should be motivated by an evaluation of the pharmacological mechanism and the risks in relation to ST.

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Deprescribing cardiometabolic medication from the perspective of the patient

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Introduction

There is increasing attention for deprescribing in older patients. Because of their preventive nature, medication for cardiometabolic risk factors can be an important target for deprescribing. Little is known, however, about the patients' perspective on deprescribing such medication.

Objectives

To identify barriers, facilitators and willingness of patients to deprescribe cardiometabolic medication, and to explore the role pharmacists can play in this process according to patients.

Methods

Two focus group interviews were held with 8 and 10 patients/caregiver. Included were Dutch speaking patients of at least 70 years, using at least 5 chronic medications including cardiometabolic medication. The interviews were recorded and transcribed verbatim. The results were analysed using directed content analysis by 2 researchers.

Results

Some patients did not see the need to stop any of their cardiometabolic medication. They attributed their wellbeing to their medication. Other patients were willing to stop some of this medication. They expressed negative feelings towards their medication, partly because of experienced or potential side-effects. Those willing to stop still considered some of their cardiometabolic medication as essential. Which medication was considered essential differed per patient and was linked to their

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beliefs about the medication or the disease. Patients expressed most trust towards their prescribing physician to handle deprescribing. Initiation by a pharmacist was not preferred by most patients. Patients who received a medication review in the past were more positive about initiation by their pharmacist, as long as the prescribing physician would be involved.

Conclusions

The patient's perceptions regarding the benefits and risks of their cardiometabolic medication and the involvement of the prescribing physician are important factors to consider when proposing to deprescribe.

ABS-66449102

Incorporating the frequency of (generic) drug switching in the analysis of related adverse drug reactions, in The Netherlands

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Introduction

Switching between bioequivalent drug products is not expected to lead to Adverse Drug Reactions (ADRs). Nonetheless, the Netherlands Pharmacovigilance Centre Lareb receives ADR reports related to generic substitution regularly. Analysis of these ADRs is hampered due to the lack of data on the population at risk.

Objectives

The aim of this investigation was to put the absolute number of ADRs related to drug substitution as reported to the Lareb in perspective of the number of drug product switches, to study whether this would lead to a difference in peak identification of the ADR report rate or not.

Methods

We performed a retrospective cohort study in the Dutch patient population using the number of drug product switches obtained from the National Health Care Institute in the Netherlands and the number of ADR reports which was obtained from the Lareb, for a selection of 20 drugs, for a period of 7.5 years (mid 2009-2016).

Results

In total, we collected data on 23,872,960 drug product switches and 1,348 ADR reports for the 20 included drugs. The average rate at which ADRs were reported in our dataset is 5.7 per 100.000 switches, but there was high temporal variability as well as per drug. Relatively high average report rates were observed in our study period for rivastigmine, levothyroxine, methylphenidate and ethinylestradiol/levonorgestrel (74.9, 50.9, 47.6 and 15.1 per 100.000 switches respectively). Analysed per quarter year, notable report rate peaks were observed for rivastigmine, levothyroxine, methylphenidate and salbutamol. We demonstrated that if the number of switches is not incorporated, thus based on the

absolute number of ADRs alone, in 10 quarter years (1.7%), the observed peak would be false positive and in 21 quarter years (3.5%) falsely negative. Additionally, we demonstrate similar results when relating the number of ADR reports to the number of drug users instead.

Conclusion

We demonstrate that in the analysis of drug substitution related ADRs it is important to include the number of drug product switches as this leads to a difference in peak identification of the ADR report rate. The number of users could be a surrogate for the number of switches.

ABS-66194583

Dysgeusia - a prodromal symptom of anaphylaxis following parenteral drug administration?

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Introduction

Medicinal products administered orally frequently induce chemosensory side effects, including dysgeusia which is a distortion of the sense of taste. Such effects have also been described for medicinal products administered parenterally. In a recent guideline dysgeusia ('metallic taste') was described as a minor prodromal symptom of anaphylaxis¹.

Aims

To investigate if there is an association between adverse reactions dysgeusia and anaphylaxis following parenteral drug administration.

Methods

Summary of Product Characteristics (SmPCs) of innovator medicinal products (defined as new active substances) approved by European Medicines Agency (EMA) between 2014 and 2018 were assessed. Products administered by injection and which reported in section 4.8: "Undesirable effects" of the SmPC dysgeusia and anaphylaxis were identified. For anaphylaxis the search criteria included the terms 'anaphylaxis OR anaphylactic OR anaphylactoid AND/OR hypersensitivity'. The European public assessment reports (EPARs) were checked for the inclusion of dysgeusia within the document.

Results / Conclusions

Between 2014 and 2018 there were 310 innovator medicinal products authorised by EMA of which 119 (38.4%) were administered via injection. Dysgeusia was an adverse reaction that was reported for 20/119 (16.8%) of the injectable products. Reports of dysgeusia were accompanied by reports of anaphylaxis in 19/20 (95%) of cases.

The identified products targeted blood disorders (7/19), cancer (5/19), infections (3/19), diabetes (2/19) and schizophrenia (1/19), and one product was used for radionuclide imaging (1/19). Without access to clinical databases of the identified products it is not possible to ascertain whether these adverse reactions occurred in the same individuals in the same time frame, however the high frequency (95%) of simultaneous reporting suggests that there might be an association.

Despite the inclusion of dysgeusia in section 4.8 of the SmPC, sometimes even with a frequency of 'common', dysgeusia was mentioned in only 9/19 EPARs. This suggests that it is often not considered to be a relevant side effect or associated with anaphylaxis.



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Identification and acknowledgement of prodromal signs, including dysgeusia, might be of value to clinical research, but also in clinical practice.

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Association between individual cholesterol and albuminuria response and exposure to atorvastatin or rosuvastatin

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Objective

The PLANET trials showed that atorvastatin 80 mg (ATOR) but not rosuvastatin at either 10 or 40 mg (ROSU) reduced urinary albumin:creatinine ratio (UACR) while effects on LDL cholesterol were similar. However, individual changes in both UACR and LDL cholesterol to these statins varied widely between patients. This interindividual variability could not be explained by patients physical or biochemical characteristics. We assessed whether the plasma concentration of the statins were associated with LDL cholesterol and albuminuria response.

Design, setting and patients

The PLANET trials randomized patients with an urine protein:creatinine ratio of 500 – 5000 mg/g, fasting LDL cholesterol >2.3 mmol/L and stable treatment with ACE or ARB to a 52 week treatment period with ATOR 80 mg, ROSU 10 mg or 40 mg. For the current analysis available samples on week 52 from therapy adherent patients (>80% compliance by pill count) were included (N=295). Plasma concentrations of ATOR and ROSU and of active metabolites were measured by Liquid Chromatography Mass Spectrometry.

Main Outcome Measurement

Percentage change in UACR and absolute change in LDL cholesterol (delta LDL), comparing baseline to week 52.

Results

Median (interquartile range) plasma concentrations at week 52 for ATOR 80 mg was 2.8 (1.7 – 8.5) in the Atorvastatin group; for ROSU 10 mg 0.7 (0.6 – 1.8) and ROSU 40 mg 2.5 (1.9 – 6.6) in the Rosuvastatin group. The variation in plasma concentration of the statin was (weakly) associated with the LDL changes and not with UACR changes for both statins (table). Serum albumin ($\beta = 0.63$, $p = 0.05$) and eGFR per 10 ml/min ($\beta = -0.09$; $p = 0.04$) were independently associated with ROSU plasma concentration. Active metabolites concentration of either ROSU or ATOR did not correlate with LDL and UACR changes.

Conclusions

Individual variation in plasma concentrations of both atorvastatin and rosuvastatin explained partly the LDL changes of the patients. The individual variation in albuminuria effects of ROSU and ATOR were not explained by the level of plasma concentration of statin or its metabolites.

Table: Pearson correlations between plasma concentration ATOR and ROSU and change in LDL cholesterol and UACR

	Delta LDL		Delta UACR	
	Pearson Correlation	P-value	Pearson Correlation	P-value
Atorvastatin (n = 92)	-0.20	0.06	0.19	0.07
Rosuvastatin (n = 203)	-0.25	0.0006	0.00	0.94

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Real-world outcomes of metastatic renal cell carcinoma treatments from electronic health records: results of a text-mining approach versus manual collection

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Introduction

Real-world data (RWD), are necessary to complement data from randomized clinical trials on the outcomes of new oncologic therapies¹. The standard collection method of collecting RWD from electronic health records (EHR) is by manual review, which is time-consuming and error-prone². Recently, CTcue clinical data collector (CDC) a software package assisting search through EHR, has become available.

Aims

The aim of this study is to compare RWD of five treatments of metastatic renal cell carcinoma (mRCC) using CTcue CDC versus manual review.

Methods

Queries were designed in CTcue CDC to identify the correct patient population and to collect patient characteristics at the start of treatment and treatment outcomes of patients with mRCC, treated with cabozantinib, pazopanib, sunitinib, everolimus or nivolumab. Patients were included between January 2015 until May 2019 in the Leiden University Medical Center. The same variables were manually collected. Categorical variables were compared and accuracy was calculated. Continuous variables were compared with Bland Altman plots.

Preliminary results

Of 95 patients identified with CTcue CDC 91 patients (95%) matched the manual population of 97 patients. Within those 91 patients, 161 (98%) of 165 matching treatments were found. Categorical variables (such as comorbidities) showed an accuracy from 61% to 94% and continuous variables (such as progression-free survival) revealed a mean difference of up to 5%.

Conclusion

Using CTcue CDC 95% of patients who were otherwise manually detected were identified. Also, the analysis of patient characteristics showed a moderate to high level of agreement. Thus, CTcue CDC is a promising text mining tool for collecting real-world data.

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Limited association between serotonin (5-HT) receptor gene polymorphisms and treatment response in antidepressant drug-naïve patients with depression

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Major depressive disorder has become a prominent cause of disability, as lifetime prevalence has increased to ~15% in the Western world¹. The pharmacological effects serotonin (5-hydroxytryptamine, 5-HT) are mediated through 5-hydroxytryptamine receptor (HTR) binding. Serotonin regulation of amygdala activity is attained through activation of three 5-HT2 family receptor subtypes, 5-HT2A, 5-HT2B, and 5-HT2C². Specifically, HTR2A and the HTR2C receptors have similar gross cerebral distribution and function, with higher constitutive activity found in HTR2C than in HTR2A.

Aims

To investigate the possible association of 5-HTR gene polymorphisms to specific and non-specific antidepressant treatment responses in treatment-free patients in Siberia.

Methods

152 patients, aged between 18-70 years and clinically diagnosed with depressive disorders, were treated with antidepressants for four weeks. DNA was genotyped for a subset of 29 SNPs from the following 5-HT Receptor genes: HTR1A, HTR1B, HTR2A, HTR2C, HTR3A, HTR3B and HTR6. Primary outcome was measured by differences in Hamilton Depression Rating Scale (Δ HAM-D) scores between baseline/week two, week two/week four and baseline/week four. Linear regression and ANOVA determined the significance between polymorphisms and Δ HAM-D.

Results / Conclusion

Over the course of the four weeks, the total Δ HAM-D for the patients was 19.2 ± 5.5 , with the Δ HAM-D in the first two weeks, 11.3 ± 4.6 and the latter two weeks, 7.9 ± 4.5 . Multivariate linear regression analyses over the three time periods was conducted for Δ HAM-D between the factors found improved Δ HAM-D in patients taking tricyclic antidepressants (Δ HAM-D 0 – 4 weeks: $B = 4.40$, $p = 0.003$; Δ HAM-D 0 – 2 weeks: $B = 3.36$, $p = 0.010$) compared to patients taking SSRIs. Genotype association with Δ HAM-D was identified in HTR1A rs6295, rs749099, HTR1B rs6298, rs6296 and HT2A rs1928040. However, further ANOVA studies for the aforementioned SNPs did not find significance between alleles.

Acknowledgement

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ABS-66449300

The use of antidepressants and anxiolytics among COPD patients in the Netherlands

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Chronic obstructive pulmonary disease (COPD) is often accompanied by psychiatric symptoms, such as depression and anxiety, affecting both treatment outcomes and mortality. Depressive symptoms are even more common in COPD than various other chronic diseases. However, the number of COPD patients using medication for these disorders is not well known.

Aims

To give an overview of the antidepressant (AD) and anxiolytic (ANX) drug use among COPD patients compared to subjects without or with other chronic diseases.

Methods

The NControl database containing prescription data of 800 pharmacies including 7 million individuals in The Netherlands was used. Patients of age 55+ that received frequent prescriptions for COPD-medication ($n=96,302$), statins ($n=422,344$), oral glucose lowering medication (OGL; $n=165,953$), dermatological drugs ($n=62,863$) and disease-modifying antirheumatic drugs (DMARDs; $n=7899$) between 2013 and 2018 were analyzed for concomitant chronic use of ADs and ANXs. We also analyzed four control groups of subjects aged 55+ ($n=734,843-3,196,443$) who were not included in any of the above patient groups.

Results/Conclusions

In general, patients that receive frequent medication for chronic diseases have a significant higher risk of using ADs or ANXs chronically than others. 20.3% Of patients that receive COPD treatment, 21.3% of patients that are treated for dermatological problems, 17.9% of patients that receive DMARDs, 15.4% of statins users and 14.9% of OGL users are also treated for depression or anxiety, compared to 3.7%, 7.5%, 12.8% and 13.9% in control groups. Except for the dermatological patient group, AD and ANX use was significantly higher in the COPD patient group compared to the other patient groups, suggesting that psychiatric symptoms are more common in COPD than in most other chronic diseases.

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ABS-66226142

Dapagliflozin stabilizes the tubulointerstitial fibrosis marker Dickkopf-3

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Aims

Urinary Dickkopf-3 (DKK3) is a stress-induced tubular epithelial-derived profibrotic glycoprotein that induces tubulointerstitial fibrosis through its action on the canonical Wnt/ β -catenin signaling pathway. A previous study showed that DKK3 concentrations are higher in patients with CKD than in the general population, and that a rise in urinary DKK3 was associated with significant eGFR decline. Prior experimental and clinical studies have suggested that SGLT-2 inhibition may reduce renal fibrosis. We therefore assessed the effect of the SGLT-2 inhibitor dapagliflozin on urinary DKK3.

Methods

24hr urine samples were used from a double-blind, randomized, placebo controlled crossover trial in 31 patients with type 2 diabetes and albumin:creatinine ratio (UACR) >100 mg/g on a stable dose of an ACE inhibitor or angiotensin receptor blocker. Patients were assigned to 6-week treatment periods with dapagliflozin 10 mg/d or placebo in random order. Urinary DKK3 was measured by ELISA at the start and end of each 6-week treatment period. A mixed effects repeated measures model was used to assess the effect of dapagliflozin on urinary DKK3.

Results/Conclusions

Dapagliflozin decreased UACR by 43.9% (95%CI: 30.3 to 54.8) and eGFR by 5.1 (2.0 to 8.1) mL/min/1.73m² compared to placebo. At baseline, urinary DKK3 concentration was 574.8 [1st, 3rd quartile: 304.3, 1223.7] ng/24hr. After 6 weeks placebo treatment, urinary DKK3 levels increased by 41.7% (95%CI: 2.2 to 96.4), $p=0.0373$, whereas they remained stable after dapagliflozin treatment (-1.2% (-29.3 to 38.2), $p=0.9421$). Accordingly, dapagliflozin lowered DKK3 compared to placebo by 30.3% (2.0 to 50.3), $p=0.0384$. After dapagliflozin, change in urinary DKK3 was significantly correlated with change in UACR ($r=0.41$, $p=0.0309$). No correlations with changes in other clinical markers (HbA1c, eGFR, SPB, Hb, Hct) were observed.

In conclusion, dapagliflozin stabilized urinary DKK3 after 6 weeks of treatment, while an increase was observed during placebo treatment, suggesting that dapagliflozin may lessen tubular stress and fibrosis. Future studies of longer treatment duration and clinical outcomes are needed to confirm the clinical impact of these findings.

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Demographic and clinical factors that impact values attached to drug effects: a preference study among type 2 diabetic patients

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About 50% of patients with type 2 diabetes do not reach their glucose (HbA1c) treatment targets. Incorporating patient preferences at the time of prescribing may improve treatment satisfaction, adherence, and subsequently, outcome. Preferences may be influenced by patient- and disease-related characteristics.

Aims

To evaluate to what extent demographic and clinical factors influence the importance patients attach to certain drug effects.

Methods

A cross-sectional survey was administered to adult patients with type 2 diabetes in The Netherlands and Turkey who received ≥ 1 prescription of an oral anti-diabetic drug in the last 4 months. The hypothetical anti-diabetic agents were described by six attributes: HbA1c decrease, cardiovascular risk (CVR) reduction, weight change, gastrointestinal (GI) adverse drug events (ADEs), hypoglycaemic events and bladder cancer risk (BCR). A multinomial logit models with treatment attribute - patient characteristic interactions were fitted separately for each of the demographic and clinical factors.

Results

The 381 patients (199 Dutch and 182 Turkish) were on average 60 (SD 10) years old, 45% were male, mean BMI was 29 (SD 5) and 35% were higher educated. Mean diabetes duration was 9 (SD 7) years and 19% of the patients reported experience with ADEs. The interaction analyses revealed that drug preferences varied strongly between country and age (see table). Turkish patients attached more importance to reducing CV risk compared to Dutch patients, who preferred to maintain body weight and not having GI ADEs, and hypoglycaemic events. Younger patients attached more importance to reducing CVR and no increasing BCR, while older patients preferred to maintain body weight and not having GI ADEs. Experience with ADEs, sex, BMI, and diabetes duration were marginally associated with drug preferences. Education did not show any effect on drug preferences.

Weights given to the different drug attributes by interaction term

Drug attributes	Country		Age	
	Turkey	Netherlands	Younger	Older
HbA1c reduction	0.11	0.14	0.07	0.09
CV risk reduction	0.51	0.20	0.61	0.23
Weight change	0.06	0.15	0.00	0.16
GI side effects	0.11	0.23	0.06	0.30
Hypoglycaemic events	0.16	0.22	0.16	0.19
Increased risk of cancer	0.05	0.06	0.10	0.02

Conclusions

Demographic and clinical factors seem to have an effect on patient preferences. The observed heterogeneity should be acknowledged when prescribing drugs and patient and disease characteristics could be a starting point to elicit such preferences.

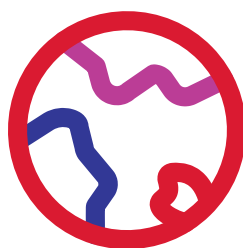
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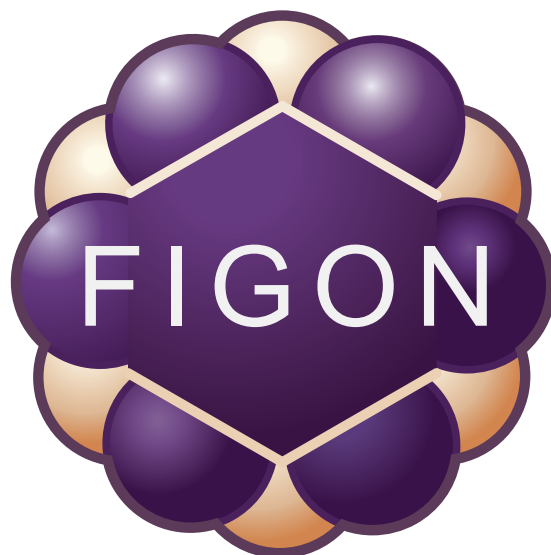
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