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Abstracts

Medicines shortages; why, who, what, how?

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Each of us have probably experienced some type of non-availability of a medicine. The availability of authorised medicines is a prerequisite for a well-functioning healthcare system, and naturally, increasing number of shortages is one of the major concerns among health authorities across the world. Any attempt to understand the root causes of shortages reveals a complex picture of a global supply chain. Various stakeholders seem to have very different – sometimes contradictory- perceptions of the actual root causes. Thanks to the Covid-19 pandemic, this global challenge has also attracted political attention leading to increased eagerness to find solutions and to be better prepared.
This presentation will explore some major root causes of shortages as we know them today and give an overview of the initiatives at national and EU level to combat the problem. The complexity of the problem calls for more research in the field to help the authorities find sustainable solutions.

Targeting Aryl Hydrocarbon Receptor for Cancer Treatment and Improved Immunotherapy

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Immunotherapy, a type of cancer therapy that targets the immune system, has recently shown unprecedented clinical outcomes. One of the most studied types of treatments is inhibition of the immune checkpoint proteins, programmed death receptor 1 (PD1) and its ligand, PDL1, which when engaged deactivate cancer killing T cells. Unfortunately, not all patients respond to immunotherapy, supporting the need for new treatment options. The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor that was initially identified as a key protein in the mediating the toxicity of environmental contaminants. Because of this, its potential as a therapeutic target was largely disregarded. AHR is now recognized to be an essential gatekeeper that integrates dietary, environmental, and endogenous signals to modulate immune cell homeostasis and promote immune suppression. AHR also exhibits cancer autonomous activity displaying tumour-specific pro-oncogenic and tumour suppressor-like functions which depend on context- and tumour-type. However, the immunosuppressive actions of AHR allow tumour cells to “hide” from immunosurveillance, suggesting that AHR inhibition would activate immune cells to better target cancer cells. We and others are currently pursuing the suitability of inhibiting AHR alone or in combination with immune checkpoint inhibitors to improve cancer therapy. Recently, the AHR target gene, poly-ADP-ribose polymerase 7 (PARP7; also known as TIPARP) has emerged as a potential anti-cancer therapeutic. PARP7 functions as a negative regulator of AHR but also as a negative regulator of type I interferon (IFN-I) signalling. Inhibition of PARP7 restores IFN-I signalling resulting in tumour regression in cancer models. These anti-tumour activities are due its effects on the immune system but also the proliferation of cancer cells. In this presentation, I will discuss the potential of AHR and PARP7 as anti-cancer therapeutics and provide insight into our studies on the roles of the AHR-PARP7 signalling axis in tumorigenesis.

Økotoksikologi

EGGTOX: Increased understanding of the mechanistic effects of crude oil toxicity during early life stages of cold-water fish

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Crude oil impacts multiple processes in the developing organism and may result in adverse outcomes for the organism. The development and use of Adverse Outcome Pathways (AOPs) are important in a risk assessment process. Developing AOPs means understanding how exposure to oil components affect the organism and triggers a cascade of detrimental events such as reduced survival or growth. Increased knowledges of how and which oil components affect various processes in the organism gives us a better ability to predict long-term effects.

Crude oil is a complex environmental mixture. To identify which components, or fractions of the oil are causing cardiac toxicity, we exposed early embryonic stages of Atlantic haddock (*Melanogrammus aeglefinus*) to both single oil components and oil fractions. We also exposed early stages of Atlantic haddock, Atlantic cod (*Gadus morhua*), Atlantic halibut (*Hippoglossus hippoglossus*), Saithe (*Pollachius virens*) and Polar cod (*Boreogadus saida*) to crude oil to evaluate species specific sensitivities. By using a frequent sampling regime, we aimed to evaluate genetic markers and link them more strongly to the outcome for the organism. The downstream effects of cardiac function on eye development were further assessed by dissecting 3-day-old haddock larvae that had been exposed to either whole crude oil, a cardiotoxic oil component, or a known blocker of cardiac function.

The project has provided an increased understanding of how oil pollution affects the organism, which can differ among species. Furthermore, the results demonstrate that the serious detrimental effects of oil pollution cannot be attributed to individual components or individual fractions of the oil, and that mixture effects have a strong role.

Atlantic cod receptor-based reporter bioassays for assessing environmental toxicity of sediments, effluents and chemicals - outcomes from the dCod 1.0 project

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Among the goals of the dCod 1.0-project was to develop methods that can be useful in assessing the health of the Ocean in various contexts, e.g. as tools for environmental monitoring and risk assessment. In the Adverse Outcome Pathway (AOP) framework, the molecular initiating event (MIE) represents the binding of the toxicant to its target, often a receptor inside a cell or on the cellular surface. MIEs lead to a cascade of downstream key events (KE), which can result in an adverse outcome, i.e. toxicity. The aryl hydrocarbon receptor (Ahr) and the nuclear receptors (NRs) comprise important MIE targets for environmental pollutants. These receptors are able to bind and be activated by a wide range of xenobiotic chemical compounds, acting as xenosensors, and as transcription factors their modulation may lead to physiological perturbation representing endocrine and metabolic disruption.

Knowledge about the specificity and sensitivity of xenosensors in wildlife in general, and in key indicator species in particular, is an important aspect of ecotoxicological research. This knowledge is also the basis for developing receptor-based bioassays for use in environmental monitoring and toxicity testing. The Atlantic cod (*Gadus morhua*) is a fish species of high importance in pelagic and coastal ecosystems of the North Atlantic, as well as in Norwegian fisheries. It is also a widely used indicator species in environmental monitoring programs in Europe.

In the dCod 1.0 project (2016-2021) we have cloned and characterized the two cod Ahrs as well as a number of cod NRs. For each receptor, we have established a luciferase gene reporter assay (LRA), where single compounds, mixtures, and environmental extracts have been tested. Using this battery of LRAs, we have shown how 11 different bisphenol A (BPA) analogs present similar, and sometimes stronger, endocrine disrupting properties, by activation of the cod estrogen receptor (Er) and androgen receptor (Ar). Using extracts from sediments representing a gradient of pollution from the inner parts of Byfjorden in Bergen to relatively pristine reference sites, we observed strong receptor activation at the more polluted sites. In the PW-exposed project, we are characterizing the toxicity of produced water from petroleum production using an effect-directed analysis (EDA) approach with fractionated extracts, pinpointing specific fractions with the highest Ahr activating potency for chemical identification. A similar fractionation and EDA approach has been initiated with the most polluted urban sediments in an ongoing MSc project. Furthermore, effluents from sewage treatment plants in Bergen have been tested in our receptor bioassays, indicating potential biological effects in the recipients.

Together, our results demonstrate that a battery of receptor-based bioassays are useful in environmental monitoring and toxicity testing, at the same time advancing our knowledge of environmental risk from various complex pollution sources.

The studies were funded by the Norwegian Research Council through the projects dCod 1.0 (248840), iCod 2.0 (244564), and PW-exposed (280511).

Toksikologi

A bronchial cell culture model to investigate toxicological effects of particulate matter

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Background

Air pollution is considered as one of the main causes of early deaths. Diseases linked to air pollution include respiratory infections, chronic obstructive pulmonary disease, ischemic heart disease, lung cancer and stroke. The respiratory system is the first target of exposure and substantial work has been performed to develop advanced 3D lung models to investigate effects and mechanisms of action of various types of air pollutants. 3D cellular models, consisting of two or more different cell types, is considered to be closer to simulate a real organ and can be useful alternatives to animal studies.

Methods

Human epithelial (Calu-3), endothelial (EaHy926), immune cells (THP1) and liver cells (HePG2) were cultivated according to recommended procedures. The endothelial and epithelial cells were seeded om transwell insert. In a tri-culture model differentiated THP1 macrophages was infiltrated with the epithelial cells. The day before use the epithelial cells were put at air liquid interface (ALI) conditions. In some models, liver cells and THP1 monocytes were seeded on the basolateral side of the insert to simulate exposure to secondary organs. The co-cultures were exposed at ALI for aerosolized particles in a Vitrocel exposure system. Cell viability (alamar blue and LDH), cytokine formation (ELISA) and CYP expression (qPCR) were analyzed.

Results

The 3D lung models at ALI were vulnerable to desiccation when cultivated om 0.4 µm membranes, therefore 1 µm membranes were chosen. A high trans epithelial resistance (TEER) was established indicating development of gap junctions. Lactate dehydrogenase accumulated in the culture medium during cultivation, which encourage frequent medium shifts. Exposure to diesel particle induced CYP expression both in epithelial and endothelial cell in the coculture as well as in secondary exposed HepG2 liver cells. Only minor effects on cytokine expression and formation were observed, which can be attributed to the long exposure time of 24 h.

Conclusions

Advanced lung models with a mix of cell types can be useful for the investigation of the effects of airborne particles and chemicals on the lung and enable assessment of interactions within a multicellular environment. The complexity of a 3D co-culture model raises, however, attention on how the different cell types interact, their resistance against desiccation when at ALI and other features such as type, and volume used of culture medium. Preliminary experiments indicate that the 3D lung model is suitable for the investigation of airborne particles, such as permeation through the epithelial and endothelial membrane barrier, and effects on secondary exposed cells.

Økotoksikologi

Intrinsic clearance efficiency of the three-dimensional rainbow trout (*Oncorhynchus mykiss*) hepatocyte model when assessing three different fragrance chemicals

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Introduction and Aim

Hydrophobic chemicals which are not biotransformed have the potential to bioaccumulate in tissue and lipid reservoirs of aquatic organisms such as fish, causing both short and long-term biological effects. The assessment of a chemical's potential to bioaccumulate in fish requires determination of its physicochemical properties (e.g. log Kow) as a screening method or conventional animal (*in vivo*) test methods (OECD TG 305). Due to ethical and economical concerns, the development of alternative methods to measure *in vitro* biotransformation rates in primary hepatocytes (RT-HEP) and S9 fractions (RT-S9) from rainbow trout were established (OECD TG 319A/B) to improve existing *in silico* predictions. Due to the limited lifetime of RT-S9 and RT-HEP, these assays are not suitable to measure very slowly biotransformed chemicals. Three dimensional hepatic spheroids from rainbow trout (RT-SPH) with a longer assay duration has recently developed. The aim of this study was to determine the biotransformation rates of three different fragrance chemicals (log Kow 4.5-5.1) using RT-SPH, one slowly (Cashmeran, CASH), one moderately (Ambrofix, AMB) and one rapidly (Cyclohexyl salicylate, CS) biotransformed in the TG 319 A/B assays. The RT-SPH viability was measured during the full period of incubation (0-72 h) and decrease of the parent chemicals in active and heat-inactivated spheroids analysed by GC-MS. The *in vitro* intrinsic clearance rates ($CL_{IN\ VITRO,INT}$) were compared amongst RT-SPH, RT-S9 and RT-HEP.

Results

The RT-SPH were viable and a log-linear depletion was obtained during the whole period of incubation (up to 72 h) for all chemicals tested. Around 50% of the initial amount of CS, AMB and CASH was biotransformed between 4 and 48 h. Decrease in the heat-inactivated control was negligible (<20%) for all three chemicals. The $CL_{IN\ VITRO,RT\text{-}SPH}$ rates of AMB and CS were 5- and 25- fold lower, compared to the RT-HEP $CL_{IN\ VITRO,INT}$. CASH, which is slowly biotransformed in both RT-S9 and RT-HEP, displayed a similar CL rate in the RT-SPH. However, extrapolation to the *in vivo* whole body biotransformation rate (K_B) displayed the RT-HEP and RT-S9 (0.05 and 0.06/d, respectively) underestimated the *in vivo* K_B (2.11/d) which was derived from the BCF by a factor of ca. 35-40, whereas RT-SPH did so by a factor of 5 (0.43/d).

Conclusion

The RT-SPH are highly metabolically competent for at least 72 h and may better reflect the *in vivo* scenario when measuring compounds with slow CL rates.

Økotoksikologi

A binary mixture of PFOS and PFOA elicits a potentiating effect on the peroxisome proliferator-activated receptor alpha 1 (PPARa1) from Atlantic cod (*Gadus morhua*)

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Per- and polyfluoroalkyl substances (PFAS) are a group of man-made compounds that have been widely used in consumer and industrial products. Perfluoroalkane sulfonic acids (PFSA) and perfluoroalkyl carboxylic acids (PFCA) are two major classes of PFAS, which include perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), respectively. Many PFAS molecules possess both bioaccumulating and biomagnifying properties, and have recently been detected in marine teleosts, including Baltic cod and Atlantic cod. Furthermore, it has been shown that some PFAS can bind and activate peroxisome proliferator-activated receptors (PPARs), which have a major role in the regulation of lipid- and carbohydrate metabolism in vertebrates. Using an *in vitro* luciferase reporter gene assay, we have found that Atlantic cod PPARa1 was transactivated by the carboxylic acids PFHxA, PFOA, PFNA, as well as the sulfonic acid PFHxS, while PPARa2 was not activated by any of these compounds. Homology modeling, molecular docking and molecular dynamics simulations (MD) of PFOS, PFOA, PFNA and PFHxA complexed to the PPARa1 and PPARa2 ligand-binding domains (LBD) supported the experimental data, suggesting that PFHxA, PFOA and PFNA more significantly stabilized the omega loop region of the LDB in PPARa1 than in PPARa2, while PFOS did not stabilize this region in neither subtype. Intriguingly, a binary mixture of PFOS and PFOA produced both a higher activation of PPARa1 and lowered the EC_{50} compared to the activation by PFOA alone. Ligand docking analyses of double-ligand complexes identified a putative allosteric binding site situated near the ligand binding pocket and the omega loop in the AF-2 region. Subsequent MD simulations of the gmPPARa1 complex revealed that the hydrophobic fluorocarbon chain locates to a hydrophobic pocket, and that binding of PFOS in this alternative binding site leads to further stabilization of the omega loop region, leading to a more structurally stable ligand binding domain and, in particular, the region near the coactivator binding site. Thus, although not active by itself, binding of PFOS to this second allosteric binding site gives rise to an unforeseen interaction effect that potentiates gmPPARa1 activity, putatively by stabilizing an active conformation of the receptor. Thus, exposure of Atlantic cod to PFAS individually, or in mixtures, could potentially modulate the lipid- and carbohydrate metabolism by directly interfering with PPARa1. Importantly, also PFAS that do not act as PPARa agonists may modulate receptor activity via binding to allosteric binding sites.

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Toksikologi

Mechanisms of carcinogenic potential of occupational exposure to manufactured nanomaterials

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Telomers are protective structures that are important in preventing genome instability. Telomere shortening can result in cellular senescence and in increased level of genome instability, which is in turn a key event in numerous of cancer types. Despite this, a limited number of studies have focused on the effect of nanomaterial exposure on telomere length as a possible mechanism in cancer development.

Method
In this study, effects of long-term exposure to multi-walled carbon nanotubes (MWCNT) on telomere length were investigated in mice exposed by intrapleural injection and in the lung epithelial and mesothelial cell lines. In addition, cell cycle, apoptosis and regulation of genes involved in DNA damage repair were assessed.

Results
Pleural injection of the MWCNT, Mitsui-7 and NM-401 led to infiltration of inflammatory cells in the pleura as well as mesothelial cell hyperplasia. These histological alterations were accompanied by deregulation of genes involved in fibrosis and immune cell recruitment, as well as a significant shortening of telomeres in the pleura and the lung. Assessment of key mechanisms involved in development of cancer in vitro confirmed that long-term exposure to the long MWCNT NM-401 led to telomere shortening in epithelial cells, which was coinciding with G1-phase arrest and enhanced apoptosis.

Conclusion
Altogether, our data show that telomere shortening resulting in cell cycle arrest and apoptosis may be an important mechanism in long MWCNT-induced fibrosis and consequent carcinogenesis.

Environmental toxicology

Using fin whale fibroblasts in toxicological studies

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Background

Fin whales (*Balaenoptera physalus*) and other marine mammals can be exposed to and affected by persistent environmental toxicants that accumulate and biomagnify throughout the food chain. Studies show the presence of several organic toxicants in these animals (1,2). The goal of this study is to improve the knowledge about toxicological responses in fin whale by studying fin whale fibroblasts.

Methods

Firstly, two different culturing conditions were tested to find optimal growth conditions for the fibroblast cells. Secondly, the cells will be used in exposure experiments with various environmental toxicants and toxicant cocktails. Lastly, we will attempt to reprogram the cells into mesenchymal stem cells (MSCs) using the MSC inducing cocktail of Lai *et al.*[3] and further differentiate them into other cell types (e.g., adipocytes).

Results

The two growth conditions tested were (1) adding basic Fibroblast Growth Factor (bFGF) to the culture medium and (2) growing the cells on a collagen coated surface. Adding bFGF showed a marked increase in growth rate. The cells hit full confluency several days before the controls. Collagen coating had no apparent effect on growth rate, the cells hitting confluency around the same time as the controls. Exposure of the cells to benzo[a]pyrene has been done and western blotting and qPCR have been done for cytochrome P4501A1 (CYP1A1) protein and mRNA analysis. The western blot showed no increased synthesis of CYP1A1, and the qPCR results currently shows no increased transcription of the *cyp1a1* gene. Cells induced into MSCs showed a morphological change. However, qPCR analysis with genes specific to MSCs showed no increased transcription of the genes.

Conclusion

Adding bFGF to the culture medium gives better growth conditions for the fibroblast cells. Collagen coating showed no significant improvements. The cells seem to only grow for about 6–8 passages. There was a morphological difference between reprogrammed cells and the control, though there are no concrete data supporting that change did happen.

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Toxicology

Sustainable cereal production reduces important *Fusarium* mycotoxins

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Background
Mycotoxins in cereals constitute major problems for animal and human health worldwide. *Fusarium* moulds attack growing cereals species and are considered the most important source of mycotoxins such as deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin and HT-2 toxin in small grains in temperate climates.

Method
Available knowledge on the impact of production systems, organic versus conventional, and the influence of specific agronomic parameters on the occurrence and concentrations of DON, ZEA and T-2/HT-2 in wheat, oats, barley and rye are presented. The agronomic factors associated with *Fusarium* mycotoxin risks are discussed in the context of the needs for sustainable cereal production.

Results
Most studies of acceptable scientific quality that compared mycotoxins in organic and conventional cereal production reported lower *Fusarium* mycotoxin concentrations in organic compared to conventional cereals. Specifically, 24 comparisons reported lower mycotoxin level in organic production, 16 detected no significant difference, whereas only two comparisons found higher level in organic production. When the mean concentrations of DON, ZEA and T-2/HT-2 from all studies were compared, conventionally produced cereals had 62, 110 and 180 % higher concentrations than organic cereals. Previous studies suggest that diverse crop rotations, high soil organic matter content and microbial/biological activity are associated with lower *Fusarium* mycotoxin concentrations, whereas high mineral nitrogen fertiliser, specific fungicides, herbicides and tillage are not means that seem to reduce the risks of *Fusarium* mycotoxin levels in cereals.

Conclusion
The management of *Fusarium* moulds and mycotoxins requires a preventative, integrated and holistic agronomic approach.

Toxicology

Loss of AHR reduces pancreatic cancer cell migration

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Introduction: Pancreatic cancer is one of the most aggressive and deadly solid malignancies with few therapeutic options. A potential therapeutic target is the aryl hydrocarbon receptor (AHR). AHR is a ligand-activated transcription factor that has been historically studied due to its ability to mediate the toxicity of environmental pollutants. However, AHR drives pro-survival processes that increase tumour growth, while its immunosuppressive actions allow tumor cells to “hide” from immunosurveillance. Thus, inhibiting or loss of AHR activity would be expected to reduce tumour growth and increase immune cell mediated tumor killing. The aim of the current study was to examine the effect of AHR loss or its inhibition on the proliferative and migratory properties of pancreatic cancer cells. Our long-term goal is to study AHR loss on tumour growth in immunocompetent mouse models.

Methods: We used the mouse pancreatic cancer cell line, K8484, which was derived from a spontaneous pancreatic tumor from LSL-**K**ras$^{G12D/+}$;LSL-Tr**p**53$^{R172H/+}$;Pdx1-**C**re (KPC) mouse model; a genetically engineered mouse model of pancreatic cancer. We also used BxPC3, a human pancreatic cancer cell line. AHR^{ko} cells of both cell lines were generated using CRISPR/Cas9 gene editing. DNA sequencing, RT-qPCR and western blotting were used to confirm AHR knockout. Cell proliferation was determined using IncuCyte and xCELLigence instruments, and cell migration was measured by a scratch assay, this has been completed for the K8484 cells.

Results: DNA sequencing confirmed the introduction of “indels” resulting in the incorporation of a premature stop codon in the AHR mRNA. Lack of AHR expression was verified by western blotting. AHR knockout reduced cytochrome P450 1A1 (CYP1A1) basal activity and prevented AHR ligand induced CYP1A1 levels in both BxPC3 and K8484 cells. AHR loss had no effect on the proliferation of K8484 cells. However, K8484 AHR^{ko} cells migrated significantly less than wildtype (WT). The characterization of the BxPC3 AHR^{ko} cell line is ongoing.

Conclusion: Loss of AHR reduces migration of K8484 cells. Although more studies are needed, these findings suggest that AHR inhibition may be a potential therapeutic strategy for late stage metastatic pancreatic cancer.

Toxicology

The DNA-binding domain of the Aryl hydrocarbon receptor is vital for protection against chemically induced colitis in mice.

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Introduction
The aryl hydrocarbon receptor (AHR) is a transcription factor best known as a mediator of the toxic responses of environmental pollutants. AHR, however, is now recognized as an essential gatekeeper integrating metabolic signals to promote immunosuppression, regulate immune cell function and inflammation. In the gut, the AHR functions to maintain a healthy intestinal environment by reducing inflammation. AHR regulates its target genes through direct DNA binding to aryl hydrocarbon response elements (AHRE) but also through tethering to other transcription factors in a DNA-binding independent manner, many of which regulate inflammatory responses. However, it is not known if AHR's anti-inflammatory role in the gut requires its ability to bind to AHREs. To test this, we determined the sensitivity of $Ahr^{dbd/dbd}$ mice, a genetically modified mouse line that express an AHR protein that is incapable of binding to AHREs, to dextran sulfate sodium (DSS)-induced colitis(Poland and Knutson 1982).

Methods
$Ahr^{dbd/dbd}$ mice (Bunger et al. 2008) were exposed to 2% DSS in their drinking water for 6 days before being switched to normal water and monitoring them for an additional 7 days. Body weight and disease index score was measured daily, RNA was isolated from colon tissue and changes in gene expression were measured by RT-qPCR, histological staining of the distal colon was done and inflammatory cell infiltration was determined.

Results
The $Ahr^{dbd/dbd}$ mutant mice have increased sensitivity to DSS-induced colitis compared with $Ahr^{+/+}$ mice. $Ahr^{dbd/dbd}$ mice exposed to 2% DSS exhibited severe symptoms of intestinal inflammation compared with $Ahr^{+/+}$ mice. None of the $Ahr^{dbd/dbd}$ mice survived the 2% DSS exposure. On day 6, the $Ahr^{dbd/dbd}$ mice had severe body weight loss, shortening of their colon length, higher disease index scores, enlarged spleens, and increased expression of several inflammation genes, including interleukin 1b (*Il-1b*), *Il-6*, *Il-17*, C-x-c motif chemokine ligand 1 (*Cxcl1*), *Cxcl5* and lipocalin-2.

Conclusion
Our findings demonstrate that $Ahr^{dbd/dbd}$ mutant mice are very sensitive to DSS-induced colitis and are therefore phenotypically similar to $Ahr^{-/-}$ mice in this response. Our data show that AHR's DNA-binding domain and ability to bind to AHREs is required for it to reduce inflammation and maintain a healthy intestinal environment.

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Klinisk farmakologi

Short- and long-term effects of body weight, calorie restriction, and gastric bypass on CYP1A2-, CYP2C19-, and CYP2C9 activity

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Objective: Roux-en-Y gastric bypass (RYGB) may influence drug disposition due to surgery-induced gastrointestinal alterations and/or subsequent weight-loss. The objective was to compare short- and long-term effects of RYGB and diet on the metabolic ratios of paraxanthine/caffeine (cytochrome P450 (CYP) 1A2 activity), 5-hydroxyomeprazole/ omeprazole (CYP2C19 activity) and losartan/losartan carboxylic acid (CYP2C9 activity), and cross-sectionally compare these CYP activities with normal- to overweight controls.

Methods: This trial included patients with severe obesity preparing for RYGB (n=40) or diet-induced (n=41) weight loss, and controls (n=18). Both weight loss groups underwent a 3-week low-energy-diet (<1200 kcal/day, week 0-3) followed by a 6-week very-low-energy-diet or RYGB (both <800 kcal/day, week 3-9). Follow-up-time was two years, with four pharmacokinetic investigations.

Key Results: Mean±SD weight-loss from baseline was similar in the RYGB group (13±2.4%) and diet group (11±3.9%) at week 9, but differed at year 2 (RYGB: -30±7.0%, diet: -3.1±6.3%). From week 0-3, mean CYP2C19 activity similarly increased in both groups (RYGB: 43% [95% CI: 16, 55], diet: 48% [95% CI: 22, 60]). Mean CYP2C19 activity increased by 30% [95% CI: 2.6, 43] after RYGB (week 3-9), but not in the diet group, between-group difference: -0.30 [95% CI: -0.63, 0.03]. CYP2C19 activity remained elevated in the RYGB group at year 2. Baseline CYP2C19 activity was 2.7-fold higher in controls compared with patients with obesity, whereas no difference was observed in CYP1A2- and CYP2C9 activity.

Conclusion: Our findings suggest that CYP2C19 activity is lower in patients with obesity and increases following weight loss, partly mediated by RYGB. This may be clinically relevant for drug dosing. Neither body weight, RYGB, nor weight loss had any clinically significant effect on CYP1A2- and CYP2C9 activity.

Basal farmakologi

Effects of chronic pharmacological activation of GC-B with CNP in a mouse model of heart failure with preserved ejection fraction

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Heart failure (HF) is the most rapidly growing cardiovascular health burden world wide with nearly 50% of patients suffering from HF with preserved ejection fraction (HFpEF). Although several advances have been made in the understanding and treatment of HF with reduced ejection fraction (HFrEF), the majority of HFpEF patients lack effective treatment options. We have previously shown that activation of GC-B with CNP increases cGMP in cardiac myocytes that lead to increased removal of Ca^{2+} into the sarcoplasmic reticulum, faster relaxation and phosphorylation of titin that leads to more compliant cardiomyocytes. We therefore hypothesize that CNP increases diastolic filling that could alleviate HFpEF. We therefore wanted to investigate long-term treatment with CNP in a mouse model of HFpEF. To induce HFpEF, mice were fed a high fat (HFD) diet to induce metabolic stress, and administered an inhibitor of nitric oxide synthase (L-NAME) in the drinking water to induce hypertensive stress. Mice receiving maintenance diet (MD) were included as control.

To explore whether CNP prevents development of HFpEF, subcutaneous insertion of osmotic pumps containing either CNP or vehicle were performed in HFD+L-NAME and MD mice. Preliminary results from pilot studies revealed decreased cardiomyocyte area and septum width with mice treated with CNP, indicative of hypertrophy improvement with CNP. A non-significant trend towards decreased lung weight was also noted in the group of mice receiving CNP-treatment, suggesting reduction in lung congestion.
These preliminary results could indicate that CNP-treatment is beneficial in HFpEF.

Basal pharmacology

Natural killer cell-derived extracellular vesicles as potential therapeutics for solid cancer

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Research question

Extracellular vesicles (EVs) are natural delivery vesicles, normally released by cells for intercellular communication. We are exploiting extracellular vesicles released by NK cells (NK-EVs) as potential killer nanovesicles. Our working hypothesis is that they have unique tumor-targeting characteristics inherited by the donor cells, and that they more efficiently infiltrate solid tumors due their small size. We have recently shown that bulk EVs separated from NK cells potently kill tumor targets. Here, we asked whether there is a subset of EVs released from NK cells that are particularly enriched in cytolytic molecules, and whether these would have enhanced tumor killing capacity compared to bulk NK-EVs.

Method

Primary NK cells or the NK cell line NK-92 were cultured under resting or activated conditions to induce EV release. Protein content of the EVs was mapped via LC-MS/MS. Tumor cell death was measured as Caspase 3/7 cleavage, monitored in tumor spheroids via live monitoring using the IncuCyte technology. EV subsets were generated by density gradient ultracentrifugation or size-exclusion chromatography. Both separation methods yielded 3 main fractions containing vesicles, and Western blotting confirmed presence of canonical EV markers. Essential cytolytic molecules responsible for tumor death was pinpointed via a series of shRNA knockdowns of donor NK cells.

Results

Primary NK cells or NK-92 cells generated EVs with comparable ability to induce apoptosis of spheroids generated from a panel of human colon, melanoma, glioblastoma, prostate, breast, and ovarian tumor cell lines. Importantly, NK-EVs internalized into the tumor cells, and were also able to infiltrate the tumor spheroid core resulting in apoptosis. The mechanism for interaction was shown to partly involve engagement of the NKG2D ligands MICA/B expressed by sensitive tumor cells. Proteomic analysis indicated similar distribution of cytolytic proteins in EVs derived from primary NK cells or NK-92 cells. However, the analysis indicated that the EV isolate likely contained a heterogeneous mixture of vesicles derived from different intracellular sources. To address whether we could further enrich a subset of cytolytic EVs, we performed extensive proteomic profiling of EV subsets isolated through density gradient ultracentrifugation or SEC. A subset of EVs enriched in cytolytic proteins were identified, and that were more potent in inducing tumor cell apoptosis than bulk EVs.

Conclusion

We propose that a subset of cytolytic EVs derived from activated primary NK cells or NK-92 cells has promising potential to infiltrate and target solid tumors.

Basic Pharmacology

Understanding novel ligand binding to the mu-opioid receptor (MOR)

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Background: The opioid system consists of four main receptor classes recognized as *μ* [mu], *δ* [delta], *k* [kappa] and the latest discovered, *nociceptin/orphanin FQ peptide* receptor (NOP). Opioid receptors are G-protein-coupled receptors (GPCRs) that can either be activated by endogenous opioid peptides or exogenous opioid compounds such as morphine or fentanyl. Despite serious side effects, analgesics that act on opioid receptors are still considered one of the best antinociception treatments. Widespread abuse of opioids has led to the emergence of a new phenomenon known as the 'opioid epidemic'.

Aim: Based on the hypothesis that central analgesia with reduced side effects is obtainable by occupying a different site in the μ-opioid receptor (MOR) ligand binding domain, we wanted to characterise several μ-selective benzomorphan agonists with a peculiar pharmacological profile to examine residues involved in their MOR binding [1, 2].

Methodology: Specific amino acids in the MOR ligand binding domain were proposed using molecular modeling. These were mutated and mutant MOR were expressed in HEK293 cells. Radioligand binding assays were performed on membrane preparations using [^{3}H]-DAMGO to determine affinity of benzomorphan derivatives.

Results: We identified five mutations in the MOR and constructed the corresponding mutant receptors. All mutant receptors bound [^{3}H]-DAMGO. The affinities of DAMGO and one of the benzomorphan derivatives was reduced in the MOR Y3.33A and MOR Y7.43A mutants, whereas morphine binding was abolished. This suggests that some of the residues could be important in the binding pocket of the benzomorphan derivatives.

References:

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Basic pharmacology

Connections between Natriuretic Peptides and Cardiac Mitochondria

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Mitochondria are essential for cardiomyocyte function, both as main sites of ATP production and regulators of vital cellular processes. In heart failure (HF), mitochondrial function is largely impaired. This dysfunction is considered one of the main contributors to HF, and warrants further studies on mitochondria as a therapeutic target.

Drugs increasing natriuretic peptides (NPs) are currently on the market for HF treatment. The NPs (ANP, BNP and CNP) act through membrane-bound guanylyl cyclases (GCs) to generate cyclic guanosine monophosphate (cGMP) in the cytosol. Cyclic GMP subsequently activates a signaling cascade of proteins, resulting in multiple beneficial effects on the cardiovascular system. Recent work from our group has revealed an NP-induced increase in cGMP around the mitochondrial compartment, as well as a protective effect in apoptosis in cardiomyocytes. Further, it is reported that NP signaling is involved in metabolism and mitochondrial processes in other tissues than the heart. This could imply a role for NPs in cardiac mitochondrial function. In this study, we investigate whether NPs increase cGMP inside the mitochondrial matrix, the mechanism of entry, and possible effects on cardiac mitochondrial function.

Experiments were performed in H9c2 cells, a rat cardiac myoblast cell line. Our results from H9c2 cells show that stimulation with ANP and CNP resulted in an increase in cGMP using a matrix-targeted cGMP biosensor and Förster resonance energy transfer (FRET) technology. The significant FRET response indicates that NP-induced cGMP is present inside the mitochondrial matrix, which could further indicate a functional effect on the mitochondria. In order to study how cGMP is increased within the matrix, we stimulated isolated mitochondria from H9c2 cells with NPs and measured mitochondrial cGMP levels by cGMP ELISA. Our preliminary data show that ANP and CNP increase cGMP levels upon direct stimulation of mitochondria. While this suggests the presence of NP receptors in the mitochondrial membrane, further investigation is needed to support this claim. Ultimately, while further experiments are needed, the findings of this study could be of great interest in the search for targets of a novel HF therapy.

Farmakologi

Natriuretic peptides, apoptosis and cardiac mitochondria

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Introduction
The Natriuretic peptides ANP, BNP and CNP activate transmembrane guanylyl cyclases (GC) that produce cGMP. Natriuretic peptides have beneficial effects in the cardiovascular system and have previously been shown to regulate energy metabolism. However, little is known about the direct effect of natriuretic peptides in cardiac mitochondria and possible effects on cardiomyocyte apoptosis.

Objectives
Determine whether natriuretic peptides increase cGMP in cardiomyocytes around mitochondria and whether this alters apoptosis.

Materials & methods
Primary rat adult cardiomyocytes were cultured and apoptosis was evaluated by TUNEL staining and PARP cleavage. The involvement of the intrinsic pathway of apoptosis was determined by cytochrome c release and caspase 9 activation. To measure cGMP, we constructed a novel FRET-based biosensor and targeted this to the outer mitochondrial membrane (OMM). We measured phosphorylation of Drp1, and used Mitotracker and confocal microscopy to evaluate mitochondria elongation.

Results
Stimulating GC-A with ANP or GC-B with CNP reduced apoptosis and PARP cleavage, together with reduced caspase 9 activation and reduced cytochrome c release. This suggests that NPs decrease apoptosis through the intrinsic pathway that involves mitochondria. Moreover, we found that ANP and CNP could increase phosphorylation of the pro-apoptotic protein Drp1 and could induce mitochondria elongation, suggesting a protective effect. We have previously shown that GC-A-stimulation only produced modest cGMP increase using an untargeted cGMP biosensor. Here, we constructed a novel FRET-based biosensor selective for cGMP and targeted this biosensor to the OMM. Stimulation with either CNP or ANP increased cGMP locally around the mitochondria.

Conclusion
The natriuretic peptides ANP and CNP are protective against apoptosis. Our results suggests that cGMP targeting the mitochondrial outer membrane microdomain inhibits the pro-apoptotic protein Drp1, leading to mitochondrial elongation that inhibits apoptosis.

Klinisk farmakologi

Impact of fasting status and circadian variation on the pharmacokinetics of mycophenolate mofetil and the glucuronide metabolite in renal transplant recipients

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Background**:** Mycophenolate mofetil (MMF) is an immunosuppressive prodrug used for the prevention of allograft rejection. After oral administration, the prodrug is rapidly hydrolyzed to the active mycophenolate acid (MPA) which is further converted to inactive glucuronide metabolite (MPAG) via UGT enzymes. The aim of the study was twofold; to investigate the impact of fasting vs non-fasting status and circadian variation and on the pharmacokinetics of MPA and MPAG in renal transplant recipients.

Method**:** Renal transplant recipients with stable graft function treated with tacrolimus, prednisolone in addition to MMF 750 mg BID were included in this single center, prospective study. Two successive 12-hour MPA pharmacokinetic investigations (morning/evening) were performed both in a fasting (±2 hours fasting rule) and non-fasting state.

Results: A total of 30 (22 men, 8 women, mean age 55 ±16 years) renal transplant recipients performed one 24-hour investigation and 14 of these repeated the investigation within one month (n=1188 MPA/MPAG concentrations). Following the morning dose there was a slower MPA absorption rate under non-fasting compared to a fasting state, but with similar AUC_{0-12}. Following the evening dose and in a fasting state mean MPA AUC was 15% lower ($p<0.05$) and the absorption rate slower compared to the morning dose ($p<0.05$). Under non-fasting conditions, mean AUC was 13% lower following the evening dose ($p<0.01$), but the absorption rate was faster compared to after the morning dose ($p<0.05$). MPAG showed a circadian variation only under non-fasting conditions with lower AUC after the evening dose ($p<0.01$).

Conclusions*:* Both MPA and MPAG showed circadian variation with somewhat lower systemic exposures following the evening dose with limited clinical relevance in renal transplant recipients. Fasting vs. non-fasting conditions affect absorption rate differently, but with similar result in AUC.

Klinisk farmakologi

Robustness of a limited sampling strategy for plasma iohexol clearance

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Background:

The area under the plasma concentration-time curve (AUC) is a clinically useful variable for drug exposure. Pharmacometric tools in combinations with limited sampling strategies (LSS) now allow for accurate prediction of AUC with generally few optimally timed samples in a dose interval. LSS may reduce cost and patient inconvenience compared to performing full AUC determinations. However, it is not uncommon for actual sample times to deviate from the optimal time-schedule.

Method:

A previously published LSS for an iohexol population pharmacokinetic model for the determination of measured glomerular filtration rate (GFR) in patients from 1-82 years of age with GFR between 14-149 mL/min was evaluated as an example (www.mgfr.no). The LSS consisted of four samples, which were evaluated for the following deviations: 10 minutes (±6 minutes), 30 minutes (±15 minutes), 2 hours (±60 minutes) and 5 hours (-120 to +1140 minutes) following administration of iohexol. We simulated 300 individual profiles based on the prior joint distribution of pharmacokinetic parameters and covariates of the iohexol model.

Results:

Model predictive performance was generally excellent across all datasets, with mean root-mean-square error of 0.44±0.13%. Deviations of ±6 and ±15 minutes at the 10- and 30-minute sample point did not increase the mean relative error in GFR (MRE-GFR). For the 2- and 5-hour sample point, prematurely sampling 30 and 120 minutes before schedule increased MRE-GFR to 14.4% and 13.2%. For the 2-hour sample, a trend of reduced MRE-GFR for 5-60 minutes delayed sampling was found. Increasing the maximum sample time from 5 to 24 hours reduced MRE-GFR to 3.1%, with a linear trend in reduction of MRE-GFR between 5-1140 minutes delayed.

Conclusion:

LSS often rely on a strict sample time schedule but are not commonly evaluated for deviations from optimal sample times. In this work, we present a generically applicable method for LSS-robustness evaluation.

Basal pharmacology

Regulation of legumain in energy metabolism and crosstalk between myotubes and differentiating osteoblasts

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Background and aims
It is known that biochemical crosstalk by secreted mediators exist between skeletal muscle and bone due to the close location of the organs. Legumain is a cysteine protease secreted from both bone-forming osteoblasts and bone-resorptive osteoclasts. Recently our research group has identified legumain in the culture media of proliferating and differentiating primary human skeletal muscle cells. Whether prolegumain can be internalized, processed, activated and regulate glucose and/or fatty acid metabolism in myotubes is not known and is the focus in this study. In addition, the effect of the endogenous legumain inhibitor cystatin E/M and conditioned media from differentiating osteoblasts on skeletal muscle cells are studied.

Methods
Myoblasts were proliferated and differentiated into multinuclear myotubes. Cells were incubated with conditioned medium containing prolegumain (from M38L cells overexpressing and secreting prolegumain) or cystatin E/M (from M4C cells overexpressing and secreting cystatin E/M) the last 2 days of the differentiation period. In addition, conditioned medium from an osteoblast-differentiating human bone marrow stromal cell line (hBMSC-TERT4) was added to the myotubes. Total protein concentration and legumain activity was measured in cell lysates, and internalization analyzed by immunoblotting. Radioactive ^{14}C-oleic acid was used to measure fatty acid and lipid turnover and ^{14}C-D-glucose to determine glucose metabolism.

Results
Preliminary results show that extracellular prolegumain was internalized and activated in human myotubes. Further, legumain activity was increased and promoted increased oleic acid uptake but no changes in glucose metabolism. In contrast, legumain activity was decreased after culturing with and internalization of cystatin E/M. Glucose uptake and oxidation were reduced after culturing myotubes with cystatin E/M. Moreover, myotubes treated with conditioned medium from osteoblasts differentiated for 14 days, showed increased glucose uptake and oxidation, but reduced oleic acid oxidation.

Conclusion
Myotubes are able to internalize extracellularly prolegumain, which is processed to active legumain and increased fatty acid uptake. Conditioned medium from osteoblasts differentiated for 14 days increased glucose uptake and oxidation in myotubes, but reduced oleic acid oxidation. Cystatin E/M reduced glucose uptake and oxidation in myotubes. In summary, a possible biochemical crosstalk is observed between osteoblasts and myotubes, which need to be further investigated.

Basal farmakologi

Pectic polysaccharides from Norwegian Angelica show potent NK cell activation

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Problem/Pharmacological relevance/Subject

With the advent of immunotherapies against a number of cancers and autoimmune diseases, there is a steady demand for novel medicines. New sources for discovery of potentially novel immunomodulatory compounds are therefore needed. Nature contains a large and diverse reservoir of novel compounds that can be exploited for their potential as new drugs, and exploring the pharmaceutical potential of medicinal plants used in traditional medicine is highly relevant.

Method

We have reviewed historical records on usage of medicinal plants in Scandinavian folk medicine back to the 17th century in order to discover plants containing potentially novel immunomodulating compounds. Water extracts from several candidate plants were screened in immune assays, and two species of Angelica showed potent activity against macrophages and NK cells. Usage of the Norwegian Angelica plants as remedies against the cold and upper airway infections can be traced back to the Viking age. There are two subspecies of this plant in Norway, *Angelica archangelica* subsp. *archangelica* found only in high-alpine regions, and Angelica *archangelica* subsp. *litoralis* found in coastal areas. Water-extractable polysaccharides were isolated from the roots of both species, and separated into one neutral and two acidic fractions. We tested TNF-α and IFN-γ production from NK cells by ELISA, proliferation capacity via CFSE proliferation assay, receptor binding via reporter cell assays, and potential changes in NK cell phenotypes.

Results

We found that the ionized fractions of both Angelica species induced NO-release from macrophages, with *A. litoralis* being slightly more potent. The effect was likely through TLR4 or TLR2. We found that the main polysaccharide fraction, as well as a pectin-enriched acidic fraction from *A. archangelica* induced both TNF-α and IFN-γ secretion from highly purified NK cells. The compounds also induced NK cell proliferation, but had limited ability to modulate NK cell cytotoxic activity. Interestingly, polysaccharide extracts from *A. litoralis* were much less potent. Knockdown studies are underway to determine the exact sugar-binding receptor mediating the effect in NK cells.

Conclusion

The historical usage of these plants against the cold and upper airway infections in combination with our discoveries of strong innate immune activating responses, could support a function in promoting viral or bacterial immune defense.

Basal Pharmacology

The cysteine protease legumain in type 2 astrocytes and Parkinson's disease

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Introduction:
Cleavage of proteins by proteolytic enzymes (proteases) is involved in various diseases characterized by inflammation, and uncontrolled proteolytic activity may contribute to disease development. Legumain, a cysteine protease (called δ-secretase in the brain) is involved in Parkinson's disease (PD), Alzheimer's disease, stroke, ischemia, amyotrophic lateral sclerosis and multiple sclerosis. PD is characterized by loss of dopaminergic neurons of substantia nigra, formation of Lewy bodies, and neuroinflammation. It has been reported that legumain cleaves the protein α-synuclein which leads to a subsequent formation of neurotoxic Lewy bodies. Moreover, preliminary data from our group suggests that legumain is present in reactive astrocytes in several disease models of neuroinflammation, including PD. Reactive astrocytes are involved in neuroinflammation and are classified as either neurotoxic (A1) or neuroprotective (A2), but whether legumain is selectively expressed in A1 or A2 is not known. Furthermore, it is not known whether increased levels of legumain in reactive astrocytes is an early event in (and possibly driving) neuroinflammation or neuroprotection, or whether it occurs later in the disease process. In this master project, cerebrospinal fluid (CSF) and brain sections from PD patients and controls, as well as astrocyte cultures are studied to evaluate the presence and significance of legumain in PD.
Methods:
The DI TNC1 cell line of type A2 astrocytes from rat were cultured and treated with conditioned medium containing prolegumain or the legumain inhibitor cystatin E/M to study prolegumain and cystatin E/M internalization, respectively. Cell lysates were analysed by total protein measurements, legumain activity measurements and immunoblotting. Legumain in CSF samples were analysed by enzyme-linked immunosorbent assay (ELISA), while PD brain sections were analysed by immunohistochemistry (IHC).
Results:
Preliminary data showed that untreated (control) rat A2 astrocytes contained active legumain. Furthermore, the cells were able to internalize and activate prolegumain, shown by increased legumain activity and a 36 kDa immunoband of mature legumain. Also, cystatin E/M was internalized and inhibited endogenous legumain activity. Legumain ELISA of CSF samples showed no difference in legumain concentration between PD and control samples. IHC of PD brain sections showed inconclusive results.
Conclusion:
Type A2 astrocytes contain active legumain and are able to internalize and activate prolegumain, suggesting that legumain might play a role in these cells. Legumain concentration in CSF from PD or control subjects were not significantly different, suggesting low secretion of legumain to the CSF of PD patients. Whether legumain has a role in PD development or disease, remains to be explored.

Basal pharmacology

Effects of skeletal muscle cells on metabolism and regulation of the cysteine protease legumain in differentiating osteoblasts

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Research question

The interplay between skeletal muscle and bone is important in regulating energy metabolism. Studies have shown that increased physical activity enhances favourable metabolic adjustments in skeletal muscle cells and is favourable for bone formation due to enhanced bone mineral density. Recent studies suggest that skeletal muscle and bone can affect each other by secreted proteins, and that disease in one organ influences the other. It is important to understand the interplay between bone and skeletal muscle to develop new drugs against diseases in these organs. Moreover, the cysteine protease legumain has been detected in conditioned media from both osteoblasts and skeletal muscle cells (unpublished data), but whether it affects cell communication is currently unknown. The aim was to study the impact of conditioned media from proliferating and differentiating skeletal muscle cells during differentiation of osteoblasts by investigating regulation of legumain, morphology and energy metabolism.

Methods

Human bone marrow derived multipotent stromal stem cells stably transfected with the human telomerase reverse transcriptase gene (hBMSC-TERT) were utilized as *in vitro* cell model. The cells were differentiated to osteoblasts for 3, 7 and 14 days and treated with conditioned media from proliferating (day 0) and differentiating human skeletal muscle cells (days 3, 5 and 7). Legumain expression was detected by immunoblotting, whereas secretion and activity were quantified using enzyme-linked immunosorbent assay (ELISA) and measurements of the cleavage of a fluorescent peptide substrate, respectively. Energy metabolism was analysed and quantified according to the substrate oxidation method, using ^{14}C–labelled glucose or oleic acid. Total protein concentrations were also measured, and morphology studied by light microscopy.

Results

Data showed that conditioned media from skeletal muscle cells caused morphological alterations in osteoblastic cells but showed no effect on legumain activity, although a slight decrease in legumain expression was observed. Metabolism studies showed that treatment with conditioned media from skeletal muscle cells during late differentiation (day 5 and 7) significantly decreased oleic acid metabolism in differentiating osteoblasts, whereas glucose oxidation seemed to be reduced after 14 days of differentiation.

Conclusion

The results indicate that there is an interplay between osteoblasts and skeletal muscle communication in oleic acid and glucose metabolism, but not in legumain regulation. Skeletal muscle cells in the late phase of differentiation decreased oleic acid metabolism but tended to decrease glucose metabolism in differentiating osteoblast.

Basal pharmacology

Development of a 3D cell-model of myotubes in culture.

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Problem statement

Skeletal muscle contributes to the whole-body energy homeostasis by readily switching between the oxidation of fatty acids and glucose, a healthy characteristic. However, the prevalence of metabolic disorders related to muscle function like obesity or type 2 diabetes (T2D) has increased in the last decades. Dysregulation of lipid metabolism has a significant impact on whole-body energy metabolism, and changing the flux in lipid metabolism could be a key to understand the impairments attributed to skeletal muscle in obesity and T2D. At present, 2D cell models have been the most used cellular models to study energy metabolism concerning obesity and related diseases. However, the transferability of the results in this type of model to *in vivo* conditions is limited. This project aimed to develop and characterize a skeletal muscle 3D cell model (spheroids) as a tool to later study impaired molecular mechanisms characteristic of metabolic disorders.

Methods

Human satellite cells were isolated and cultured in 2D 96 well plates or in 3D by natural cell aggregation in U-bottom 96 well plates. The spheroids characterization was analyzed by image analysis, luminescence and qPCR. Glucose and fatty acid metabolisms were studied using radiolabeled substrates. The functional data were normalized by protein content measured by Bradford. Morphological and statistical analyses were performed using GraphPad Prism, ImageJ, and AnaSP.

Results

The morphology analysis indicated increased spheroid compactness during the proliferation phase. However, this parameter was drastically reduced after 5-7 days of differentiation. After 10 days of culturing, the gene expressions of differentiation markers were increased, spheroid viability was not significantly modulated and hypoxia core was almost not detected. The functional data showed that the spheroids had the same glucose uptake as for 2D cells, but lower glucose oxidation. Oleic acid metabolism was also reduced in the 3D compared to the 2D cell model.

Conclusion

The spheroids had different glucose and oleic acid metabolism, however the differentiation levels and glucose uptake were similar to the 2D cells. These preliminary analyses demonstrate some initial differences that may influence the study of the metabolism and molecular parameters related to metabolic disorders in 3D cell models.

Clinical pharmacology

Modulation of energy metabolism by eicosapentaenoic acid in skeletal muscle cells from lean and obese individuals

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Introduction
Skeletal muscle represents a large part of the body mass and is a major organ for metabolism of lipids and glucose. Various forms of lifestyle interventions that influence energy conversion and energy consumption in muscle can have an impact on regulating body weight and counteracting metabolic diseases (1). With this regard, studies have shown that intake of long-chain polyunsaturated n-3 fatty acids could have positive effects on the body's energy conversion by improving cellular function and substrate metabolism in skeletal muscle cells (2).
Methods
In the present work, we have investigated the effect of the n-3 fatty acid, eicosapentaenoic acid (EPA) *in vitro* on energy metabolism in skeletal muscle cells established from obese and lean individuals. Energy metabolism in myotubes was studied using radiolabeled substrates. Protein expression was investigated using proteomics analysis.
Results
Treatment with EPA increased glucose oxidation and glucose uptake in cells from obese individuals. Moreover, treatment with EPA decreased leucine fractional oxidation in skeletal muscle cells from both lean and obese individuals indicating increased incorporation of leucine into cellular protein. EPA treatment also increased oleic acid uptake and reduced fractional oxidation in skeletal muscle cells from both lean and obese.
Conclusion
In conclusion, these results suggest that EPA treatment enhances energy metabolism in human myotubes, suggesting that n-3 fatty acid supplementation *in vivo* may be beneficial to improve metabolic and mitochondrial function.

References
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Basic pharmacology

The influence of cytohesin inhibitor SecinH3 on natriuretic peptide signalling in cardiomyocytes

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Research question

The cardiac hormones natriuretic peptides (NPs) have a significant role in cardiac function, and are considered as potential candidates for heart failure treatment. NPs increase cGMP levels by activating the two NP receptors NPR-A (ANP and BNP) and NPR-B (CNP). Previously, our group has found that CNP causes a negative inotropic response (NIR) through the activation of protein kinase G (PKG) and potentiates the cAMP signalling through cGMP-induced PDE3-inhibition. The aim of this project is to study if these effects are regulated by cytohesins, a group of proteins spatially and functionally associated with NPRs.

Methods

The expression of cytohesins 1-4 was determined in rat and mouse ventricular cardiomyocytes by Western blotting and in rat left ventricle tissue by qPCR. Interaction of cytohesins 1-4 with NPR-A and NPR-B was assessed by co-immunoprecipitation in transfected HEK293 cells. Cyclic GMP levels were measured in rat ventricular cardiomyocytes in the presence of the cytohesin inhibitor SecinH3 using an ELISA cGMP assay. Functional responses to NPs, β-adrenergic receptor (AR) stimulation and cytohesin inhibition were investigated as changes in contractility of the isolated left ventricular muscle strips.

Results

We found that cytohesin-1, -2 and -3 were expressed in rat and mouse ventricular cardiomyocytes, and that cytohesin-2 and -4 interacted with both NPR-A and NPR-B, with the strongest association found with cytohesin-4. SecinH3 increased NPR-A-mediated cGMP levels, but decreased NPR-B-mediated cGMP. In line with the decreased cGMP levels, SecinH3 reduced the CNP-induced NIR but enhanced its lusitropic effect. SecinH3 also influenced the β_1-and β_2-AR response, changing its potency and efficacy both in presence and absence of CNP. Finally, on its own SecinH3 increased dF/dTmax and relaxation time.

Conclusion

Obtained results provide new evidence of the interaction between NPs and cytohesins and support the hypothesis that cytohesins may act as regulators of the NP system in the heart.