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Abstracts

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Sponsor av NSFTs vintermøte 2024:

BCPT
Basic & Clinical Pharmacology & Toxicology

Invited Presentations

Oral presentations toxicology

Oral presentations pharmacology

Poster presentations toxicology

POSTER#	Presenter	Affiliation
1	Kleppe & Iversen	Institute of Marine Research
2	Camassa	National Institute of Occupational Health
3	Johanson	Norwegian University of Life Sciences; Veterinærhøgsko
4	Wik	National Institute of Occupational Health
5	Ahmed	National Institute of Occupational Health
6	Krapf	National Institute of Occupational Health
7	Georgantzopoulou	Norwegian Institute for Water Research
8	Noally	University of Bergen;
9	Fimreite	University of Bergen;
10	Steinshanm	University of Bergen;
11	Myhre	Norwegian Institute of Public Health

Poster presentations pharmacology

POSTER#	Presenter	Affiliation
12	Jansrud	Diakonhjemmet Hospital; Center for Psychopharmacolo
13	Oma	University of Bergen; Dept of Clinical Science
14	Liabø	University of Oslo; Dept of Pharmacy
15	Trøften Hagen	University of Oslo; Dept of Pharmacolgy
16	Nygaard	University of Oslo; Dept of Pharmacolgy

The Beito Lecture 2024 (BCPT sponsored)

How can articifial intelligence contribute to explain the exposome effects on human health?

Karine Audouze Professor in Systems biology and Bioinformatics, Université de Paris Cité, France

Computer science can be used to have a better understanding of the putative effects from various exposure on human health. While human regulatory risk assessment (RA) still largely relies on animal studies, new approach methodologies (NAMs) i.e. in vitro, non-mammalian alternative models or computer based methods are increasingly used to evaluate chemical hazards.
Here AOP-helpFinder (https://aop-helpfinder.u-paris-sciences.fr), a computer tool based on artificial intelligence (AI) will be presented. This tool allows an automatic exploration, identification and extraction of knowledge from the literature, within the aim to develop Adverse Outcome Pathway (AOP). AOP constitutes an important framework in NAMs by understanding the causal relationships between different biological events, triggered by stressors, that can lead to adverse outcomes. An application of AI combined with integrative systems biology will be given for the development of an AOP on radiation-induced microcephaly

Drugs that impair liver sinusoidal endothelial cell function, and their metabolic consequences

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Objectives: To determine the effect of medications on the function of liver sinusoidal endothelial cells (LSECs). The microcirculation of the liver – lined by LSECs – has a unique morphology that facilitates bi-directional exchange of substrates between hepatocytes and blood in the liver sinusoids. LSECs are perforated with transcellular pores known as fenestrations, and between 2-20% of their surface is covered by fenestrations, making LSECs a highly efficient ultrafiltration system. In addition, LSECs are powerful scavenger cells, daily removing gram amounts of spent biomolecules from the circulation. Reduction in LSEC function will have profound impacts on health – for example, age-related "defenestration" is an established cause of age-related post-prandial hyperlipidemia and insulin resistance.
Methods: LSECs freshly isolated from mice were cultured on fibronectin coated plates and challenged with various medications for 30 minutes at physiologically relevant concentrations. The LSECs were either fixed for examination with scanning electron microscopy (SEM) for fenestration scoring or subjected to scavenging assays.
Results: A number of medications "chemically" age LSEC to cause defenestration (reduced fenestration diameter and number) and suppress LSEC-mediated scavenging *in vitro*.
Conclusions: If these findings translate to humans *in vivo*, then this might explain some unwanted metabolic side-effects caused by certain medications. This presentation will discuss these findings and the potential consequences for metabolism in such medicated patients.

Linking chemical disruption of molting processes to survival of crustaceans

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Abstract

Molting, an essential physiological process in arthropods, is critical for growth, development, survival, and reproduction and involves the shedding and replacement of the exoskeleton. However, this process is vulnerable to disruption by chemicals stemming from various anthropogenic activities and industrial applications. Notably, the use and discharge of certain veterinary medicines in aquaculture, pesticides in agriculture, and high-volume chemicals in industrial and municipal activities can adversely affect molting in non-target species (e.g. crustaceans, insects, arachnids, collembolas and myriapods), all of which are crucial to various ecological functions.

Decades of research have progressively improved our mechanistic understanding of how these chemicals disrupt molting, leading to the development of several Adverse Outcome Pathways (AOPs). These AOPs connect molecular-level interactions of chemicals with their biological targets to adverse phenotypical effects of regulatory relevance. Although having different Molecular Initiating Events (MIEs), these linear AOPs share common molting-relevant Key Events (KEs) and Adverse Outcomes (AOs) that collectively comprise an expanding AOP network (AOPN). The AOPs display varying levels of maturity, ranging from conceptual models to those endorsed by the scientific community through the OECD AOP work program. Current efforts are focused on expanding the knowledge domain of these AOPs, developing a better quantitative understanding of events along the AOP continuum, and integrating available knowledge and data into hazard and risk assessment practices.

The presentation will introduce the principles of AOPs, showcase molting-relevant AOPs, and propose how these can be utilized to support environmental monitoring, hazard, and risk assessment of molting-disruption in crustaceans.

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Endocrine disruption in marine organisms: nuclear receptors as key mediators

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Endocrine disruption is a phenomenon where chemical contaminants interfere with the endocrine system of living organisms. Such compounds are called endocrine disrupting compounds (EDCs), and among the primary mechanisms of EDCs is the ability to mimic endogenous hormones and activate or block receptors for signaling molecules. The binding of a chemical to a receptor is a defining moment of toxicity and is defined as a Molecular Initiating Event in the Adverse Outcome Pathway (AOP) framework. Nuclear receptors (NR) are a family of transcription factors with the ability to be modulated by a diverse range of ligands, including lipids, bile acids, steroid hormones and other signaling compounds. From the initial discovery of feminized fish in UK rivers caused by the presence of estrogenic chemicals in sewage effluents to the current attention to obesogenic chemicals in food, in household and personal care products, the importance of nuclear receptors as mediators of endocrine and metabolic disruption has expanded beyond the homodimeric steroid hormone receptors in NR subfamily NR3 to include the RXRs (NR2) and the RXR heterodimers in subfamily NR1. The functional properties and the presence or absence of these receptors in the genome of a species is thus of central importance in defining AOPs available to the organism in question.

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Experimental methods to identify endocrine disruptors affecting reproductive development – opportunities and challenges

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Abstract
In mammals, sex determination is regulated by genetic factors. Subsequent differentiation of the sex phenotypes, however, is influenced by a spatiotemporally regulated hormone signaling network. During fetal life, the male phenotype develops in response to a surge in androgen levels during a critical stage in development, often referred to as the masculinization programming window (MPW). High levels of androgens are produced by the testes and released into the circulation to reach target tissues. Androgens, via androgen receptor (AR) activation, will ensure differentiation of male reproductive organs such as prostate and external genitalia, but also other male traits. In the absence of testes, as is the case in female fetuses, there is no surge in androgens and the same tissues will instead differentiate into female reproductive organs, *etc*. Importantly, hormonal regulation of sexual development is not an either-or dimension, but rather a function of ratios between different hormones such as the androgen-estrogen balance. Disruption of this hormonal balance, particularly by anti-androgenic or estrogenic substances, can therefore impact reproductive development with potential life-long consequences. This includes exposure to endocrine disrupting chemicals (EDCs). Because of this understanding, we can test chemicals for potential to disrupt reproductive development and function using numerous alternative test methods, including steroidogenesis and nuclear receptor activation assays. However, although we have a good understanding of the regulatory mechanisms governing sexual development, it remains difficult to predict *in vivo* outcomes from non-animal data, not least because of the complexity of intact organisms. Nevertheless, the integration of new approach methodologies (NAMs) and enhanced knowledge of chemical-biomolecule interactions offers opportunities to refine testing methods and decrease reliance on *in vivo* toxicity studies in chemical assessment and risk identification.

Perfluorinated compounds: Effects on nerve cells and the nervous system

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Objective:
Per- and polyfluoroalkyl substances (PFAS) is a diverse group of man-made chemicals detected in humans and wildlife with a widespread presence in the environment. They are used at a large scale in different industries and in consumer products including personal care products. Exposure to PFAS occur mainly through ingestion of contaminated food, water or dust or through inhalation. PFAS cross the blood-brain-barrier and have been detected in brain samples from both humans and animals. Although epidemiological evidence are inconclusive, adverse effects of PFAS exposure on nerve cells and the nervous system have been detected in both *in vivo* and *in vitro* studies. In our experiments we focused on effects of chemicals from the perfluorinated group of compounds on nerve cells and the nervous system, and studies were conducted with both single compounds as well as perfluorinated-containing mixtures.

Methods:
In vitro studies including cytotoxicity studies and gene-expression studies were conducted with single perfluorinated compounds as well as perfluorinated-containing mixtures in cerebellar granule neurons (CGNs) from rat and chicken, in human neural stem cells, and in PC12 cells. In *in vivo* studies from maternally exposed mice, perfluorinated compounds were measured in dam and offspring brains as well as blood. Studies in chicken embryos were conducted after injection of perfluorinated-containing mixtures into the allantois at embryonic day 13, and concentrations of compounds measured in brain. Studies in zebrafish larva were conducted after exposure to perfluorinated-containing mixtures and perfluorooctanesulfonic acid (PFOS) only.

Results:
In rat CGNs, toxicity increased with carbon chain length of the perfluorinated compound and a sulfonate functional group caused greater toxicity than a carboxyl group. PFOS-induced excitotoxicity in CGNs involved Ca^{2+} influx via the N-Methyl-D-aspartate (NMDA)-receptor. This was blocked by specific NMDA-R antagonists. PFOS was further found to potentiate glutamate signalling. When mixtures of perfluorinated compounds were combined with brominated and/or chlorinated compounds, toxicity in CGNs, as well as potentiation of lipid-peroxidation increased. Perfluorinated compounds were detected in brains from mouse dams and offspring after oral administration to dams via feed. In chicken embryos, speed of distribution of perfluorinated compounds to the brain differed from that of chlorinated and brominated compounds. In zebrafish larvae exposure to perfluorinated mixtures or PFOS only increased swimming speed, a behavioural endpoint.

Conclusion:
Our work, using several species and cell-types, indicated that perfluorinated compounds may adversely affect the nervous system.

Nivåer og maternal overføring av per- og polyfluorerte alkylstoffer (PFAS-er) i klappmyss (*Cystophora cristata*) mor-unge par fra Vestisen

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Problemstilling

Per- og polyfluorerte alkylstoffer (PFAS-er) er protein-bundne, persistente stoffer som finnes i Arktis og som kan bioakkumulere og biomagnifisere oppover i næringskjeden. Hensikten med denne studien var å se på forekomst og nivåer av PFAS-er i klappmyss (*Cystophora cristata*) fra Vestisen, og om disse stoffene overføres med melk fra mor til unge. Klappmyss har et relativt høyt nivå i næringskjeden og en intensiv die-periode (3-4 dager) med overføring av en svært fettrik melk (>60%), noe som gjør denne arten til en god modell for å studere overføring av kjemikalier gjennom morsmelk. Ingen studier er tidligere blitt gjort for å studere nivåer eller maternal overføring av PFAS i klappmyss.

Metode

Det ble tatt blodprøver av 15 mor-unge par og melkeprøver av 9 mødre i mars 2008 i Vestisen, øst for Grønland, av Norsk Polarinstitutt. Plasma og melk ble analysert for to perfluoroalkyl sulfonater (PFSA): perfluoroheksansulfonat (PFHxS) og perfluorooktansulfonat (PFOS), og 6 perfluoroalkyl karboksylsyrer (PFCA)**:** perfluorooktanoisk syre (PFOA), perfluorononanoisk syre (PFNA), perfluorodekanoisk syre (PFDA), perfluoroundekanoisk syre (PFUdA), perfluorododekanoisk syre (PFDoA), perfluorotrideckanoisk syre (PFTrDA) på NMBU. Analysemetoden inkluderer ekstraksjon, opprensing av fett og forurensninger, deteksjon og separasjon på HPLC-MS-MS. Den statistiske analysen ble utført ved bruk av statistikkprogrammet R.

Resultater

Alle stoffene det ble analysert for ble detektert i alle prøvene, bortsett fra PFHxS, som kun ble detektert i én melkeprøve. Det var høyere nivåer av PFAS i blodprøvene enn i melkeprøvene. Dette er fordi PFAS-er er proteinbundne, og typisk akkumulerer i proteinrike vev, som blod, lever og nyrer. Unge-mor-ratioer var på 3,33 (±1,01) for PFSA og 1,7 (±0,38) for PFCA, som vil si at nivåene er henholdsvis 3,33 og 1,7 ganger høyere i blodprøver fra unger enn fra mødre. PFOS var den mest fremtredende PFAS-en, og stod for 38% av total PFAS i mor- blodprøvene, 46% i unge-prøvene og 39% i melkeprøvene. Det var generelt ulike mønster av prosentandel for de ulike PFAS-ene i mor-blodprøvene, ungeprøvene og melkeprøvene. Nivåene av PFAS var innenfor spekteret av nivåer funnet i andre selarter fra Grønland (Bossi et al. 2005, Butt et al. 2010).

Konklusjon

Resultatene i denne studien viser maternal overføring av PFAS da alle stoffene som ble detektert i plasma fra mødre også ble detektert i ungene. 7 av 8 analyserte PFAS-er som ble funnet i plasma fra mor og unge ble også funnet i melk. Dette indikerer at stoffene blir overført fra morens blod til melken. Høye unge-mor-ratioer tilsier at PFAS blir effektivt

Transcriptomic characterization of 2D and 3D human induced pluripotent stem cell based in vitro New Approach Methodologies (NAMs) for developmental neurotoxicity testing.

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Objectives: There is a need to develop reliable and reproducible new approach methodologies (NAMs) for developmental neurotoxicity (DNT), particularly for closing knowledge gaps identified in the DNT *in vitro* battery (DNT IVB) intended for regulatory use.
Methods: This project is part of the EU funded projects ONTOX and PARC and aims to characterize a 2D human neural progenitor cell (NPC) model and a 3D human neurosphere model, derived from IMR90 human induced pluripotent stem cells (hiPSCs), undergoing differentiation for up to 21 days using RNA sequencing.
Results: Enrichment analysis was performed using four different databases: Gene Ontology (Biological Processes, Cellular Components and Molecular Functions), KEGG, Panther and Reactome. The most commonly enriched terms with differentiation were terms related to neurodevelopment and function. For further characterization of the *in vitro* NAMs, a selected panel of genes commonly used to identify distinct cell types were used and included genes representing neural subpopulations, maturation, synaptic transmission, motility, axon guidance, adhesion, cell cycle and endocrine receptors. Transcriptomics data reveals that gene markers for astrocytes and neuronal subpopulations (glutamatergic, GABAergic, serotonergic, cholinergic, noradrenergic, glycinergic and dopaminergic) are present in the cultures at all differentiational timepoints. A broad range of endocrine receptors were investigated, of which most were present. Both cultures consist of excitatory and inhibitory neurons as well astrocytes, both cell types maturing with differentiation however, the 3D culture seems to mature faster than the 2D culture.
Conclusions: Based on these results the 2D NPC model shows promising possibilities for contributing to close knowledge gaps identified in the DNT IVB, in particular for replacement of a rat-based assay with a human model for assessment of synaptogenesis. Overall, these results contribute to a better characterization of the 2D and 3D *in vitro* NAMs intended to close the existing knowledge gaps and improve the DNT IVB for hazard identification and characterization.

Developmental neurotoxicity of acrylamide and its metabolite glycidamide in a human mixed culture of neurons and astrocytes undergoing differentiation – a RNA sequencing study

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Objectives. Acrylamide (ACR) is formed when carbohydrate rich foods are prepared at high temperatures. ACR is a possible neurotoxicant that crosses the blood brain barrier and the placenta barrier. Knowing ACR effect on brain development and the underlying mechanism is therefore essential. Human neural progenitor cells (hNPCs) derived from human induced pluripotent stem cells (hiPSC) were used to investigate effects of exposure to the food processing toxicant acrylamide (AA) and its main metabolite glycidamide (GA) on key neurodevelopmental processes assessed by cell viability and gene expression (RNAsequencing, seq). A vision of the PARC project is to develop Next Generation Risk Assessment (NGRA) of chemicals to protect human health. This study aimed to characterize differentiation after ACR exposure and to find neurodevelopmental processes sensitive to exposure using a whole transcriptome approach. The results will be compared to a recent neurodevelopmental study from the Norwegian Mother, Father and Child Cohort (MoBa), which included 114 500 children, where acrylamide exposure was calculated based on a food frequency questionnaire answered around gestational week 17.
Methods. The NPCs were differentiated and exposed to AA and GA ($1x10^{-8}$ – $3x10^{-3}$ M) for up to 28 days. Effects on cell viability was measured using Alamar Blue™ Cell Viability assay, alterations in gene expression were assessed using real time PCR and RNAseq.
Results. RNAseq analysis show that exposure to sub micromolar levels of GA disturbed neurodevelopmental processes related to synaptogenesis, neural maturation, and neuronal outgrowth parameters. The identified differentially expressed genes were also significantly enriched in terms related to cellular responses to DNA damage stimulus following GA exposure.
Conclusion. Effects at sub or low micromolar range of acrylamide is relevant for human exposure during pregnancy via food items. Disturbance of any of these key neurodevelopmental processes in the developing brain may lead to impairment of cognitive processes in the child.

Evaluation of developmental neurotoxicity induced by pesticides in human iPSC-derived neural stem cells undergoing differentiation towards neuronal and glial cells.

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Objectives. Numerous complex biological processes take place during brain development both during pre- and post-natal phases. Human induced pluripotent stem cell (iPSC)-derived neural stem cells (hiNSCs) were used to investigate effects of exposure to an organophosphate pesticide chlorpyrifos (CPF) and its metabolite chlorpyrifos-oxon (CPF-oxon) which is a potent serum and brain acetylcholinesterase (AChE) inhibitor. This human cell-based model is fit-for-purpose evaluating developmental neurotoxicity (DNT) mimicking key neurodevelopmental processes. A vision of the PARC project is to develop Next Generation Risk Assessment (NGRA) of chemicals to protect human health. Chlorpyrifos is included in the project as a model compound for development of *in vitro* New Approach Methodologies (NAMs) intended for hazard evaluation of developmental neurotoxicants.
Methods. The hiNSCs were differentiated into a mixed sub-population of neurons and glia cells. The cells were exposed to CPF and CPF-oxon for up to 28 days. For both chemicals were used 5 different concentrations, starting from 0,0021 µM (amount of chemical found in the cord blood) up to 21 µM (equal to IC_5). Alterations in gene expression were assessed using Real time PCR (qPCR) and RNA-sequencing (RNAseq), and changes in protein markers for key neurodevelopmental processes was quantified using immunocytochemistry and High Content Imaging (HCI).
Results. Effects of CPF and CPF-oxon on neurodevelopmental processes were evaluated using endpoints anchored to common key events (KE) identified in the existing developmental neurotoxicity (DNT) adverse outcome pathways (AOPs). The HCI analysis showed the effect of CPF on neurite outgrowth, the number of synapses, BDNF protein levels, the relative proportion of neurons and astrocytes. The preliminary RNAseq results support the effect of CPFoxon on important pathways involved in the neurons subpopulation formation, neurons migration and synapses development.
Conclusion. The relevance of DNT has been evidenced by the association between exposure to environmental pollutants and neurodevelopmental disorders in children (such as learning disabilities, autism spectrum disorders, attention deficit hyperactivity disorder), which are becoming increasingly prevalent in recent years.

Can marine nutrients interact with TCDD induced reproductive toxicity? - effects on tissue levels, sperm quality and proteome in Wistar HAN rats.

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Objectives
Dioxins have been shown to affect male reproduction, and reduced sperm count has been observed in both experimental animal and human epidemiological studies. The current tolerable weekly intake (TWI) for dioxins and dioxin-like PCBs has also been based on this health endpoint. The main source of dietary exposure to dioxin is fatty seafood. Nevertheless, most dietary guidelines suggest increasing our fish intake due to its related health benefits.
Few studies have specifically addressed the combined effects of the dietary intake of fish and exposure to dioxins in experimental animal studies. The main aim of this study was therefore to assess potential interactions between dietary fish intake and exposure to dioxins in an animal model, with a particular focus on the reproductive organs.

Methods
Adolescent male Wistar HAN rats were exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and/or salmon proteins in a 2x2 factorial design. The dosage of TCDD was either non-added, or 200 ng/kg, while the proteins in the diet were either 100% casein or 50% casein and 50% freeze-dried salmon fillet. The diet was based on a western diet for rodents and was fed to the rats for approximately 90 days before termination. At termination, epididymal sperm samples were collected and analysed for sperm quality using manual counting and manual vitality scoring, and organs were sampled for other analyses. The liver was analysed for TCDD, while testes were subjected to histological analyses and analyzed for differential protein expression using LC-MS/MS.

Results
Neither TCDD, nor salmon protein, affected sperm quality at the current dose. TCDD analyses showed that TCDD had been taken up in the liver in the exposed groups, but no significant difference in accumulation of TCDD was observed between groups with or without salmon. Following proteomic analyses of testis, no differences were observed for interaction effect or for main factor diet effect. However, the main factor TCDD significantly induced differential expression of 28 proteins in testis, including some proteins related to the development of spermatogenesis and spermatid function.

Conclusion
Inclusion of salmon protein in the diet of developing rats did not affect TCDD toxicity in our study. TCDD in the diet did not affect sperm quality, however, proteins related to spermatogenesis were affected by TCDD.

Investigation of the pulmonary effects of exposure to micro- and nanoplastics using different human lung models

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Background

Health effects of occupational exposure to micro- and nanoplastics (MNPs) is largely unknown. At workplaces within plastic production and waste recycling, MNPs can occur through physical and chemical degradation processes and at high temperatures. Inhalation is the most relevant exposure route in such environments, and the workers' exposure levels to airborne MNPs remain unknown. Moreover, the translocation of deposited MNPs from the lungs to other organs and the potentially associated health consequences need to be elucidated. STAMI is contributing to this research as one of 28 partners in *PlasticsFatE*, an EU-funded project that aims to improve our current understanding on how MNPs and associated additives and contaminants affect the human health.

Methods

Our approaches include *in vitro* experiments assessing cell viability and immune responses in human alveolar basal epithelial cells (A549) and monocyte-derived macrophages (dTHP-1) co-cultures following exposure to pristine or microbially contaminated MNPs. These outcomes will also be studied in a more advanced air-liquid interface (ALI) 3D lung model consisting of A549 epithelial cells, dTHP-1 macrophages, and EA.hy926 endothelial cells. Moreover, translocation of MNPs from the lungs to other organs and immune effects will be assessed *in vivo* using mouse as a model organism.

Results

Our current results from submerged A549/dTHP-1 co-cultures show that exposure to pristine and microbially contaminated polyethylene terephthalate (PET) nanoparticles (D50 <100 nm) for 24 hours increased the expression of inflammatory cytokines (IL-1B, IL-6, IL-8, and TNFα). The effect was stronger in the presence of microbial contaminants. No change in cell viability was found.

Conclusion

We show that PET nanoparticles may induce inflammation in the airways by increasing the expression of pro-inflammatory cytokines *in vitro*. Whether the same and/or other effects are found in more advanced model systems, such as the 3D ALI lung model and in mice, remains to be confirmed in ongoing work.

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Molecular and functional characterization of the Atlantic cod (*Gadus morhua*) retinoid X receptor (Rxr) subtypes and their sensitivity to organotin compounds

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Organotins, and in particular tributyltin (TBT), were used as biocidal ingredients in marine antifouling products until their use was globally banned in 2008 due to their adverse effects on non-target organisms. Although the levels of TBT have significantly declined in seawater, TBT can accumulate and persist in marine sediments for several decades. Sediment pollution monitoring surveys have revealed a number of TBT hot spots along the Norwegian coast, exhibiting TBT levels that are comparable to the most contaminated TBT sites internationally. TBT is especially known for causing imposex in neogastropods where females develop a non-functional male-like sexual organ such as vas deferens and penis-like tissue that covers the female sexual organ. Imposex results in female infertility and severe declines in neogastropod populations occurred worldwide as a result of TBT exposure.

The mode of action underlying imposex is believed to be inappropriate activation of the retinoid X receptor (Rxr) signaling pathway causing an increase in the concentration of endogenous free retinoid that promotes development of imposex phenotypes. However, less knowledge exists on the interaction between TBT and Rxr in teleost species. Here we present the molecular and functional characterization of Rxra, Rxrb1, Rxrb2, and Rxrg from Atlantic cod (*Gadus morhua*), including the ligand activation pattern from five different organotins and the endogenous ligand 9-cis retinoic acid (9-cis RA). The Rxr subtypes demonstrated distinct tissue specific expression, as well as different activation profiles and sensitivities towards both organotins and 9-cis RA, where Rxrb1 and Rxrb2 were not activated by any of these compounds. The lack of activation of these subtypes is most likely due to a 14 amino acid extension of helix 7 in the ligand-binding domain. Moreover, RNA-seq analyses of TBTexposed Atlantic cod precision-cut liver slices shed new light on the molecular mechanisms and cellular signaling pathways affected by this legacy pollutant.

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Marma-detox

Whales and polar bear in a petri dish: decoding marine mammal toxicology through *in vitro* and *in silico* approaches

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Background:
Whales and polar bears are important mid or top predators in arctic marine ecosystems, which face sustained levels of contaminants with bioaccumulating and biomagnifying properties. Notably, Killer whales experience a significant burden of these pollutants potentially threatening their reproductive abilities. Despite the ecological importance of these species, a mechanistic understanding of contaminant responses in these marine animals is hampered by the challenges of studying them in their natural habitats. Addressing this, the Marma-detox project adopts an innovative *in vitro* and *in silico* approach to unravel the intricacies of marine mammals' toxicological defense systems.

The Marma-detox project is driven by the following key objectives:

I. Establish fundamental knowledge regarding the genomic underpinnings of toxicological defense systems in specific marine mammal species.
II. Characterize the functional properties of crucial molecular targets responsive to environmental contaminants within these species.
III. Examine toxicological responses in marine mammal mesenchymal stem cells (MSC) and fibroblast cell lines.
IV. Integrate *in silico* and *in vitro* findings with existing data on associations between contaminant exposure, biomarkers, and omics data in free-ranging animals.

Expected outcomes: The Marma-detox project aspires to yield valuable insights into the toxicological responses of marine mammals, elucidating their "chemical defensome." This defensome encompasses an array of transcription factors, transporters, and antioxidant enzymes working synergistically to detoxify and eliminate harmful compounds, including environmental contaminants. The project stands as a founding collaboration across marine mammal science, environmental chemistry, bioinformatics, and molecular toxicology. By deepening our understanding of marine mammal toxicology, the project aims to expand the frontiers of marine mammal biology, toxicology, and conservation, shedding light on the evolutionary and individual adaptations of these animals.

Expected impacts: Beyond its immediate contributions, the Marma-detox project holds broader significance. The methodologies and techniques under development have the potential for transferability to other environmentally susceptible and under-studied wildlife within the field of environmental toxicology. Thus, the project not only intends to advance our knowledge of marine mammal toxicology but also offers tools and insights applicable to the broader context of wildlife conservation and environmental health.

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Pharmaceuticals in wastewater from a hospital and through a wastewater treatment plant; occurrence and environmental risk, 2019-2022.

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Objectives
The use of pharmaceuticals is increasing in our society. After use, they are excreted into wastewater, potentially affecting aquatic organisms. The discharge of pharmaceuticals through hospital wastewaters may entail negative environmental effects. Our objective was to trace pharmaceuticals from the hospital through the receiving wastewater treatment plant (WWTP) and investigate to which degree these pharmaceuticals originated from the hospital. We also aimed to identify the pharmaceuticals that constitute the highest risk to aquatic organisms.

Methods
In this first large study in Norway since 2007, we measured the concentrations of >150 pharmaceuticals in hospital wastewater discharges, and in the influent and effluent of the receiving WWTP.

Results
Eight pharmaceuticals constituted 10-41% of the total amounts to the receiving WWTP. These pharmaceuticals (x-ray contrast agents, anaesthetics, certain antibiotics) are primarily used in hospitals. A simple risk assessment of the WWTP effluents indicated that the female sex hormones and drugs estrone and estradiol pose the highest environmental risk. The risk quotients (RQ) of the anti-inflammatory drug diclofenac and the antibiotics ciprofloxacin, azithromycin and sulfamethoxazole exceeded 1, indicating that environmental effects cannot be precluded. The lack of toxicity studies of several of the measured compounds implies that safe concentrations could not be derived. The hospitals' contribution to negative environmental effects should be further investigated.

Conclusions
Pharmaceuticals play an important role in extending and improving human health. However, certain pharmaceuticals may pose a risk of negative effects to the aquatic environment. The water framework directive has included pharmaceuticals on their watch-list, and a few pharmaceuticals have proposed environmental quality standards in the environment which when enforced will oblige a monitoring of them to assess environmental status.

The results of this study have been published (in Norwegian) (Grung et al. 2023). The collaboration between NIVA, Ahus and NRVA will continue to investigate pharmaceuticals in the hospital effluent and will provide more data in the years to come.

Litterature
Grung M, Vogelsang C, Schwermer CU, Helgerud TC, Espvik HJ, Raasok C, Gravningen KM. 2023. https://vannforeningen.no/wp-content/uploads/2023/11/Grung_Web_v2b.pdf

Molecular insights into glucocorticoid receptor interaction with dexamethasone in fish

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Objectives:
Synthetic glucocorticoids (GCs), like dexamethasone (DEX), are found in surface waters, affecting organisms by activating their glucocorticoid receptor (GR). Despite research on DEX's effects on fish, understanding the molecular interaction between GR and DEX remains nascent. Our multidisciplinary study explores this interaction at atomic and molecular levels in fish and human using *in silico* and *in vitro* approaches.
Methods:
To align and visualize the primary structure of GR from Atlantic cod (*Gadus morhua*), zebrafish (*Danio rerio*), and humans, Muscle and Jalview software was used. Three-dimensional LBD structures were then built using SWISS-MODEL. Molecular docking and dynamic simulations were carried out via AutoDock Vina and NAMD, respectively, with visualization done using PyMOL and VMD. Additionally, GR genes were cloned, and species-specific plasmids were constructed. An *in vitro* luciferase reporter gene assay assessed DEX's effectiveness and potency towards GR.
Results:
The results obtained from multiple sequence alignments revealed conserved active site residues across the studied species. In each of these species, DEX was found to occupy the canonical GR-LBD. The DEX-GR complex demonstrated significantly greater stability in humans than cod GR and zebrafish GR. This observation was further approved by conducting an *in vitro* luciferase reporter gene assay. The results demonstrated that DEX exhibited a higher potency toward the human GR than the Atlantic cod and zebrafish GRs.
Conclusion:
The species-specific properties influence the GR response magnitude to DEX. This study thereby contributes to a better understanding of the impact of GCs on organisms, paving the way for more targeted and species-specific environmental assessments.

Effect of fecal microbiota transplantation on CYP3A activity in patients with systemic sclerosis

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Objectives: Several studies suggest that the gut microbiota may influence the expression and activity of cytochrome P450 3A (CYP3A), the quantitative most important drug-metabolizing enzyme. The gut microbiota composition in patients with systemic sclerosis (SSc) seems to differ from that in healthy individuals and these patients may therefore have an altered expression and activity of CYP3A. Following fecal microbiota transplantation (FMT), the gut microbiota composition in patients with SSc may change and further lead to changes in CYP3A activity. The purpose of this thesis was twofold; firstly, to determine CYP3A activity in patients with SSc and secondly to investigate both short- and long-term effects of FMT on CYP3A activity using midazolam as a probe drug.

***Methods*:** The ReSScueCYP study was a substudy of a randomized, double-blinded, placebo-controlled trial, including patients from 18 to 85 years with SSc, experiencing gastrointestinal symptoms. Patients were randomized (1:1) to receive either FMT by Anaerobic Cultivated Human Intestinal Microbiome (ACHIM) or placebo at week 0 and 2. At week 12 all patients received ACHIM. Eight-hour pharmacokinetic investigation days were carried out at the three study visits. On the study days, 1.5 mg midazolam was administrated orally, followed by an individual dose of intravenous midazolam (2.5–7.5 mg) two hours later to determine differences in absolute bioavailability and clearance. Plasma concentrations of midazolam were determined using a validated UHPLC-MS/MS method, and a previously developed population pharmacokinetic model was used to determine pharmacokinetic parameters.

***Results**:* In total, 22 patients with SSc (57 ± 12 years) were included in the pharmacokinetic analyses. Mean absolute bioavailability and clearance of midazolam in patients with SSc were 59 ± 18% and 32 ± 10 L/h, respectively. Following FMT, there were no statistically significant differences in absolute bioavailability and clearance of midazolam neither between the two groups at any of the three study visits or over time in the ACHIM group.

Conclusion: Treatment with ACHIM did not affect absolute bioavailability or clearance of midazolam in patients with SSc, suggesting that CYP3A activity is unaltered following FMT. Due to higher absolute bioavailability, but similar clearance compared to what has been previously reported in healthy individuals, our findings indicate that intestinal CYP3A activity may be downregulated in patients with SSc.

The microbiome-derived metabolism of mycophenolic acid in renal transplant recipients

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Objectives: A large part of the considerable interindividual variability in mycophenolic acid (MPA) pharmacokinetics remains unpredictable and unexplained. MPA undergoes extensive enterohepatic recirculation, and the exposure of the secondary peak displays large variability. Bacterial β-glucuronidase enzymes are central in this process as they convert the primary metabolite of MPA, mycophenolic acid glucuronide (MPAG), back to MPA in the intestine, which then is re-absorbed and increases systemic exposure of MPA and likely enhances immunosuppression and toxicity. The effect of interindividual differences in gut microbiota on this enterohepatic recirculation and, thus, MPA dosing requirement is expected to be significant. This project aimed to develop a novel method for quantifying the microbiome-derived reactivation of MPA from MPAG and use the method to determine MPA reactivation rates in patient samples from renal transplant recipients.

Methods: To identify and quantify the effects of the gut microbiome on MPA pharmacokinetics, we developed our own in-house platform of *in vitro* human fecal lysates, followed by UPLC-MS/MS analysis for determination of the microbiome-derived metabolism of MPA. Shotgun metagenomic sequencing was used to assess the microbial taxonomy. The developed method was applied to fecal samples from a previously conducted clinical trial (MicrobioTac-MPA, REK). In total 65 fecal samples from 22 renal transplant recipients were analyzed. Fecal lysates were prepared by using bead beating and sonication as cell lysis techniques. MPAG was added to the lysate and incubated for 2 hours. Samples for drug concentration measurements of MPA and MPAG were obtained every 15 to 30 minutes. Cumulative MPA concentration as a function of time was fitted to a linear regression model, with the slope representing the reactivation rate of MPA.

Results: In the renal transplant recipients there was a 15-fold difference in MPA reactivation rates, ranging from 6-86 μM/h. For the 14 patients with paired 12-hr in vivo pharmacokinetic data and fecal samples, the MPA reactivation rate strongly correlated ($r = 0.78$, $P > 0.001$) with the degree of enterohepatic recirculation (%). The reactivation rate was significantly higher 3-6 weeks after transplantation compared to 1 year after transplantation (45.9 ± 25.3 versus 30.7 μM/h $\pm$ 8.8 μM/h, $P < 0.05$).

Conclusion: We have developed a method that quantifies the depletion of MPAG and the reactivation of MPA in lysates prepared from fecal samples. We showed that the microbiome-derived metabolism of MPAG displayed large interindividual variability in renal transplant recipients. The reactivation of MPA showed a strong association with *in vivo* MPA pharmacokinetics. These findings suggest that this metric can be used to improve individualized dosing of MPA in the future.

A polyphenol fraction from *Daphne mezereum* exert prebiotic effects and protect the gut epithelial barrier

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The gut microbiota consists of a wide variety of microorganisms and is a complex community interacting with the host. Alterations in the bacterial flora might lead to dysfunctions and increase the risk of developing a range of diseases. Data have shown that complex non-digestible carbohydrates and polyphenols might reduce the risk and incidence of these diseases by affecting gut health via microbial metabolism, the intestinal immune system, and changes in the microbial ecology. Here, we studied the effects of polysaccharides and polyphenols from selected medicinal plants in modulating the gut microbiota and the gut barrier. The chosen plants were *D. mezereum*, *A. archangelica* subsp. *archangelica*, *Hypericum perforatum*, and *Ranunculus acris*. By using an ultra-high-throughput 16S RNA gene amplicon sequencing technology a polyphenol enriched fraction from *D. mezereum* was found to promote a unique skewing of the microbiota profile with an increase of health promoting bacteria like *Clostridium butyricum*, *Faeacalibacterium prausnitzii* and *Bifidobacterium*, and reduction in assumed opportunistic pathogenic bacteria like *Clostridium perfringens*, *Enterococcus*, and *Pseudoflavonifractor*. The same fraction induced unique skewings of the gut metabolome. The polyphenol fraction of *D. mezereum* also showed protective effects of organoids using a 3D intestinal organoid model system. In conclusion, exploiting the polyphenolic compounds of *D. mezereum* could be a strategy to modulate the gut microbiota for promoting increased barrier integrity and dampening of immune activity.

Exploring NK cell culture conditions for optimal production of extracellular vesicles for cancer therapy

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Natural killer (NK) cells are immune cells important for killing cancer cells. Recent research has shown that extracellular vesicles (EVs) produced by NK cells can be a promising treatment strategy against cancer. With this study, we aim to identify optimal serum-free culture conditions of NK cells that yields maximum viability coupled to maximum yield of functional EVs with tumor-targeting capacity. To this end, we have cultured the NK-92 cell line in 6 different serum free media under static or shaking conditions for 48 hrs. We observed that NK-92 cells were more viable when cultured under shaking conditions. There were also clear differences in the performance of the different medium types. We are currently testing and comparing the EV yield, their morphology, and capacity to induce tumor cell apoptosis. The data will be important for future exploitation of NK-EVs for therapy, and may also be important for identifying optimal culture conditions for NK cell therapeutics.

Combining cyclic nucleotide scavengers and biosensors to decipher signaling compartmentation in cardiac cells

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The natriuretic peptides ANP, BNP and CNP activate transmembrane guanylyl cyclases (GC) that produce cyclic GMP (cGMP). We have previously shown by employing targeted FRET-based biosensors that these natriuretic peptides have differential effects in cardiomyocytes and intact heart, where CNP activates GC-B that increases cGMP near both troponin I (TnI) and phospholamban (PLB) that translates into a lusitropic response (enhanced relaxation) in heart muscle preparations. Activation of GC-A with ANP or BNP modestly increases cGMP only near PLB and do not elicit a lusitropic response, suggesting spatial cGMP signalling from GC-A and GC-B in cardiomyocytes. By targeting a novel biosensor to the outer mitochondrial membrane (OMM), we have found that both GC-A and GC-B increase subcellular cGMP at the OMM that results in reduced apoptosis of cardiomyocytes. To understand the spatial kinetics of cGMP signalling from the GC-A and GC-B receptors, we combined targeted variants of the cGMP scavenger SponGee with our targeted FRET-based biosensors for cGMP in cardiac H9c2 cells.

Activation of GC-B increased cytosolic cGMP and this was modestly reduced by the lipid raft (Lyn-SponGee), non-raft (SponGee-Kras) or OMM-targeted (OMM-SponGee) cGMP scavengers compared to the untargeted SponGee. Increases in cGMP from activation of GC-A, on the other hand, were reduced similarly by all SponGee examined. At the OMM, the localized SponGee-Kras and OMM-SponGee reduced both content and the kinetics of cGMP propagation from activation of GC-B.

Our results indicate that GC-A and GC-B are differentially organized on the plasma membrane and that cGMP reaching the OMM could have different origin. Using cGMP scavengers can therefore be used to decipher the signaling from different areas of the plasma membrane to various subcellular locations in cardiac cells.

DSSLepR obese rats as a new animal model of heart failure with preserved ejection fraction (HFpEF) for testing new pharmacological treatment strategies

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Heart failure (HF) represents a major societal and personal burden, with prevalence estimated at 1-2% in the general population and increasing with age to about 10%. Furthermore, life expectancy is dramatically shortened for those affected. Several advances have been made in the understanding and treatment of the most studied form of HF, called HF with reduced ejection fraction (HFrEF), formerly also known as systolic HF. However, almost 50% of HF patients suffer from HF with preserved ejection fraction (HFpEF), also known as diastolic HF, which remains poorly understood and lacks effective treatment options. The main problem in HFpEF is a stiff or non-compliant heart, often characterised by concentric remodelling, resulting in a small ventricular lumen causing reduced ventricular filling and thus reduced cardiac output despite preserved ejection fraction. Research on HFpEF has been hampered by the lack of good animal models. To this aim, we are currently breeding and characterising a new rat model of HFpEF. This model combines two of the main triggers of HFpEF: hypertension and metabolic syndrome and is obtained by crossbreeding rats prone to hypertension (Dahl salt-sensitive (DSS)) with rats prone to metabolic syndrome (Zucker rats (LepR): harbouring a missense mutation in the leptin receptor gene). We have established this new rat model, designated DSSLepR, in our lab and want to characterize the model to determine whether it recapitulates true HFpEF.

DSSLepR rats were obtained by initial crossbreeding of heterozygote male Zucker rats, heterozygous (fa/+) for the fa allele causing leptin resistance (LepR) with a female Dahl salt sensitive (DSS) rat, followed by nine rounds of back-crossing to ensure a stable genetic background of DSS with heterozygosity of the fa allele. Mating of such heterozygotes yields three genotypes but only two phenotypes: homozygous (fa/fa) obese, heterozygous (fa/+) lean and homozygous (+/+) lean. The obese animals were approximately 40% (male) and 100% (female) heavier than their lean littermate controls at 5-19 weeks of age, and displayed cardiac ventricular hypertrophy, assessed by transthoracic echocardiography. In addition, the obese animals had increased blood pressure and also the heart weight and lung weight were increased. Echocardiography revealed no reduction in fractional shortening but decreased diastolic compliance (E/é). Also, direct measurements of left ventricular function showed increased end-diastolic pressure-volume relationship indicating an impaired relaxation and stiffening of the heart. We therefore conclude that the animals suffer from a diastolic dysfunction. In addition, we observed increased fibrosis in the DSSLepR obese hearts, supported by increased myocardial profibrotic markers, such as collagen I and III and alpha smooth muscle actin.

In addition to further characterisation, future studies will focus on investigating the effects of neurohumoral regulation in hearts with diastolic dysfunction and HFpEF. The long-term objective is to utilise this rat model to study factors that lead to HFpEF development and to develop new pharmacological therapy for HFpEF.

Dissecting the heterogeneity of extracellular vesicles from NK cells as cancer nanotherapeutics

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NK cells release extracellular vesicles (EVs) in response to cytokine stimulation or receptor engagement. We previously showed that we can separate EVs that derive from the plasma membrane (microvesicles) from internally derived vesicles (exosomes) via density gradient ultracentrifugation. While multivesicular bodies are the main source of exosomes, we find that also cytolytic granules contain intraluminal vesicles. We show by electron microscopy that these vesicles bear the tetraspanin NKG7. NKG7 is preferentially expressed in cytolytic granules of NK cells and T cells NK-92 cells lacking NKG7 contain fewer intraluminal vesicles within cytolytic granules, and EVs from the NKG7 knockout cells are less efficient at inducing tumor cell apoptosis compared to EVs from NK-92 wild-type cells. NKG7+ EVs isolated by affinity-based capture induce apoptosis of cancer cell lines. Further, we find a striking enrichment of miRNAs 29b-3p, miR-142-3p, miR-181a-5p and miR-630 within the NKG7+ EVs. that could play a role in induction of cancer cell apoptosis. Target prediction analysis suggested that miRNAs enriched in cytotoxic EVs and $NKG7^{+}$ EVs converge on genes related to apoptosis and TGF-β receptor signaling, and we further show that the NKG7 EV subpopulation reduce expression levels of *BCL2*, *RICTOR* and *TGFBR1* in the HCT-116 colon cancer cell line, indicating that cytotoxic NK-EVs can target cancer cells through miRNA-mediated modulation of anti-apoptotic pathways. In conclusion, NKG7+ EVs may be a specialized subset of EVs involved in killing of tumor cells, and will be further exploited as potential therapeutic agent.

Effect of marine nutrients on the toxicity of selected persistent organic pollutants

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Objectives

The main objective of this study was to investigate potential interaction effects of marine nutrients on the toxicity of selected persistent organic pollutants (POPs), including 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD), dioxin-like polychlorinated biphenyl 126 (PCB-126), and a mix of pollutants (TCDD, PCB-138, PCB-153, PBDE-47, PBDE-99) associated with seafood. Due to their lipophilic properties, these contaminants accumulate in lipid rich tissues of marine animals, such as muscle tissue of fatty fish. Although fatty seafood is one of the largest exposure sources for these contaminants, a diet containing seafood is beneficial according to The Norwegian Directorate of Health. Therefore, it is relevant to investigate whether marine oils can influence the toxicity of TCDD, PCB-126 and the mix composition, with focus on physical parameters, such as body weight and organ weight, and reproductive system in male mice.

Methods

In a fractional factorial design, male c57BL/6 mice were randomly assigned into 21 groups and given either standard feed (control), low dose contaminant feed and high dose contaminant feed, as well as marine oils, over a period of 13 weeks to assess the effects of chronic dietary exposure to the contaminants. Body weight development and hematological parameters were monitored throughout the whole trial period. After euthanasia, organs were weighed, sperm was isolated from the epididymis and the number of sperm cells was counted.

Results

Groups given high dose TCDD showed a lower body weight development, lower feed efficiency and bigger liver weight compared to control and PCB groups. The sperm count did not show significant differences between the groups.

Conclusion

Preliminary results showed no effects of marine oils on the toxicity of TCDD and PCB-126. However, high dose TCDD and high dose mix showed effect on body weight and liver weight, but not on the total sperm count. Future work will entail: complete sperm quality analysis, histology assessment on liver and testis, gene expression analysis, hormone analysis and more.

An in vitro 3D advanced lung model for hazard assessment of nanomaterials on human health

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Objectives:
The aim of this study was to use an in vitro 3D advanced lung model (Camassa et al., 2022), which is resembling the in vivo histology of airway tissue, for hazard assessment of nanomaterials (NMs). This exposure model can be used as tool in occupational health monitoring. Advanced in vitro models are needed to pass from hazard assessment based mainly on animal studies to the application of new approach methodologies (NAMs).
Mehtods:
The 3D lung model consists of human epithelial cells A549, human differentiated monocytes dTHP-1 and human endothelial cells Ea.hy926, cultivated at the air–liquid interface (ALI) on the apical (A549/dTHP-1) and basolateral (EA.hy926) sides of a microporous insert membrane (1 μm).
3D lung models were exposed to 20 μg/cm^2 NM-300K (20 nm) and to 1 and 8 μg/cm^2 of two nano-CeO_2 (3.5 and 50 nm) in an aerosol exposure system (VITROCELL® Cloud-Chamber).
Before the exposure, nanoparticles were characterized by dynamic light scattering (DLS) and by scanning transmission electron microscopy (SEM).
Results:
No endotoxin was detected in the nanoparticles during testing. Hydrodynamic diameters (Z-Ave; nm) of the particles are larger than the primary size. As shown by SEM micrographs, CeO_2 nanoparticles (only excluding NM-300K) appears to aggregate after nebulization.
24 hours post exposure, both cytotoxicity and genotoxicity were measured respectively by alamarblue and comet assay. When compared to the unexposed control, cell viability was significantly affected on apical and basolateral side of the insert by NM-300K. This effect was shown by low dose (apical side) and high dose (both sides) of the smallest CeO_2. No significant effect was observed towards internal exposed controls (1:10 PBS and particles dispersant TMAOH). No DNA damage was observed. To further investigate inflammation and oxidative stress, these results will be supplemented with gene expression data by a customized RT^2 profiler PCR-array.
Conclusion:
The 3D lung model is demonstrated to be compatible with several different endpoints.

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Effect of single and combined exposure to polycyclic aromatic hydrocarbons and N-nitroso compounds on the plasma metabolome in A/J Min/+ mice

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Introduction and objectives

The consumption of processed meat has been linked to an increased risk of colorectal cancer. Several components are proposed as causes to this carcinogenic effect, including the presence of polycyclic aromatic hydrocarbons (PAHs) and N-nitroso compounds (NOCs) (Bouvard et al. 2015). However, methodological bias in epidemiological studies has been seen (Händel et al 2020), and experimental studies are needed to elucidate possible mechanisms contributing to the link between processed meat and cancer. Studying alterations in the metabolome (metabolomics) in response to carcinogen exposure can provide a better understanding of underlying endogenous mechanisms (Liu et al 2022). Moreover, alterations in the overall metabolic profiles are direct indications of changes in gene expression or enzyme activities, which further reflect the physiological state of the organism (Patti et al 2012). The present study aimed to investigate dose-dependent effects on plasma metabolite composition of NOCs and benzo(a)pyrene (a PAH) in A/J Min/+ mice, a human relevant model for colorectal cancer.

Methods

A/J Min/+ mice were divided into 5 groups (N=20 per group, both genders): Control, NOC Low, BaP (benzo(a)pyrene) Low, NOC+BaP Low and NOC+BaP High. All groups were fed an experimental gel diet (nocturnal exposure) 3 days/week from 4 to 13 weeks of age. The Low doses were calculated from human estimated daily intake levels (95^{th}-$97{,}5^{th}$ percentiles x10 000), and the High doses were x100 to the Low concentrations. Plasma was extracted for targeted metabolomics analysis using the AbsoluteIDQ® p400 HR kit and high-resolution mass spectrometry. Data var analyzed by ANOVA multivariate orthogonal partial least squares analysis and metabolites important for a significant factor (group, sex or group*sex) were identified.

Results

The targeted metabolomics kit quantified up to 365 lipids and 43 small molecules. The BaP Low and the NOC+BaP High diets significantly changed the plasma metabolic profiles compared to Controls. Interestingly, the NOC+BaP Low dose did not affect the metabolome, indicating an antagonistic interaction between the Low doses of BaP and NOCs. The results further show disruption of the glycerophospholipid metabolism, dysfunction of the sphingomyelin cycle and alterations in amino acid metabolism. Lastly, sex was a significant factor in all models indicating gender-specific compositions of the analyzed metabolites.

Conclusion

BaP and NOCs interacted antagonistically on the plasma metabolome and affected several metabolic pathways in A/J Min/+ mice. Further studies should include analysis of enzyme induction or activity to facilitate the interpretation of mechanistic effects.

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Air quality in dental clinics – characterization of particles and bioaerosols and toxicological effects on cells of the lower airways: Dentex

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Objectives. Dental health care personnel such as technicians, assistants and dentists have an increased risk of health problems related to the lower airways. While the underlying mechanisms are not well established, it is known that exposure to air borne particles in other settings is associated with for instance asthma, allergy, COPD, fibrosis, and pneumoconiosis.

New generation dental filling materials, composites, consist of a methyl metacrylate mesh and silica-zirconium filler particles. During abrasive dental procedures, sub-microscopic pieces of composite material can be released into the air, containing composite filler particles and tooth material as well as bioaerosols such as viruses, bacteria, and fungi.

The current study aims at determining levels of aerosol exposure in dental clinics, as well as detailed characterization of the detected aerosol particles in terms of size and types. Furthermore, we seek to evaluate toxicological effects of composite particles in representative experimental models of the airways both *in vitro* and *in vivo*.

Methods. The field study in dental clinics utilize both stationary and personal air sampling equipment suited for real time measurements of particle concentration levels and size as well as measurements of personal exposure levels for each individual over the work day. Furthermore, composite particles are generated by abrasive drilling under controlled conditions in the lab, and the respirable fraction collected. A 3D lung cell culture containing epithelial alveolar type II like cells, macrophages and endothelial cells will be exposed at the air-liquid interface (ALI), and biomarkers of inflammation and fibrosis measured by standard molecular biology techniques. *In vivo* studies aim to validate findings from the *in vitro* biomarker studies.

Results. Preliminary results indicate that aerosol exposure levels in modern, well-ventilated dental clinics are low. However, certain procedures may have produced more particles and bioaerosols.

Conclusion. While the current field study showed low levels of aerosol exposure, these measurements were performed in a centralized, modern facility mainly visited by referred patients which need specific medical attention. An extended field study should be performed in private clinics with a work environment more representative for most dental health care personnel.

Innate immune responses of Alternaria toxins in vitro: Receptor activation, inflammation induction, and signal transduction

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Objective: Mycotoxins are toxic secondary metabolites produced by fungi (*Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*) and pose a significant concern in the realm of food safety, occupational and public health (Aichinger et al., 2021, Solhaug et al., 2016). Exposure (i.e. inhalation, dermal, ingestion) to mycotoxins in industries such as compound feed mills, farming, and animal husbandry is prevalent and an occupational health concern (Straumfors et al., 2015). Certain mycotoxins have been described to be immunosuppressive by inhibiting the triggering effect of lipopolysaccaride (LPS) on the NFKB-signaling pathway, that is necessary to engage the immune system (Del Favero et al., 2020). This study investigates the immunotoxic effects of Alternaria toxins—specifically Alternariol (AOH) and Alternariol monomethyl ether (AME)—in an in vitro model using innate HEK-293 TLR reporter cells (TLR) and NFkB activation.

Methods: Experimental parameters involve exposing HEK-293 TLR reporter cells, encompassing HEK null, TLR2, and TLR4, to varying concentrations of mycotoxins (i.e., AOH- 30, 6, 1.2, 0.24 µM; AME-10, 2.5, 0.625, 0.156 µM), with dimethyl sulfoxide (DMSO) as vehicle control. A TLR4 ligand, ultra-pure Lipopolysaccharides from Escherichia coli 0111:B4 (LPS), and a TLR2 ligand, ultra-pure Lipoteichoic Acid from Bacillus Subtilis (LTA) were used to activate Toll-like receptors. Assessment methods include Alamar Blue for cell viability and Quanti Blue assays for detecting and quantifying TLR activity (Liu et al 2019).

Result: LPS and LTA activation of TLR receptors led to an increase in the NFκB signal. However, combination exposure of LPS and LTA with mycotoxins reduced the increased NFκB signal in a dose-dependent fashion in both receptors (TLR2 and TLR4) for both mycotoxins (AOH and AME) compared with the positive control with a significant decrease at the highest concentration for TLR2 when exposed to AME and TLR4 for both AME and AOH. Some effect of exposure to LPS/LTA and mycotoxins on the cells viability was also seen though no significant reduction.

Conclusion: The results indicate an immunoinhibiting effect of AOH and AME mycotoxins on LPS- or LTA-induced activation of TLR via the downstream NFκB signaling pathway.

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Closing data gaps on natural toxins effect on human health:
Immunotoxic effects of Alternaria toxins using a co-culture lung exposure model

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Objectives

Traditional toxicity test methods are time consuming, costly and require a high number of animals for testing, often with uncertain transferability. The Partnership for the Assessment of Risk from Chemicals (PARC) has been developed to address current, emerging, and novel chemical safety challenges associated with innovating chemical risk assessments using Next Generation Risk Assessment (NGRA) towards a toxic-free environment.
Mycotoxins, from the *Alternaria* species, is an emerging challenge with major gaps in hazard data. Thus, Alternaria toxins are part of the PARC prioritized chemicals. The species of fungi *Alternaria* are widely distributed in nature and known to infect a large variety of edible plants. The mycotoxins can be found in food and feed products as well as in organic dust derived from infected sources, putting workers from several occupations at risk through inhalation or dermal contact. Animal and in vitro studies have shown a broad spectrum of toxic effects including acute-, geno- and cyto-toxicity, developmental and foetus toxicity and immunomodulating effects, after exposure to Alternaria toxins. Certain mycotoxins have been described to be immunosuppressive by inhibiting the triggering effect of lipopolysaccharide (LPS), an outer membrane component of bacteria, on the NFKB-signalling pathway, that is necessary to engage the immune system.

Methods

We aim to use NAMs to investigate the respiratory immunotoxic effects of Alternaria toxins using the human 3D mucociliary lung tissue model (EpiAirwayTM, Mattek). The model will be complemented with seeding of differentiated THP-1 cells on the apical side of the epithelium. EpiAirway^{TM} transwell inserts will be exposed to mycotoxins in the Air Liquid Interface (ALI) system. The co-cultures will be exposed to single and mixtures of toxins (with or without co-exposure to lipopolysaccharide (LPS)), before collecting the cells for transcriptomics analysis. Pathway analysis with a particular focus on pulmonary immunotoxicity will be carried out.

Conclusion

The results may add in the development of a battery of NAMs needed for carrying out NGRA of Alternaria toxins. And, gained knowledge may be important for future setting of health-based occupational exposure limits for Alternaria toxins.

Environmental impacts of emerging nanomaterials: *in vitro* investigation of molybdenum disulfide (MoS_2) and layered double hydroxides (LDHs) using a zebrafish liver cell line.

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Objectives. Molybdenum disulfide (MoS_2) and layered double hydroxide (LDHs) nanosheets are very promising novel materials for use in environmental remediation among other applications. During production, use and disposal, these materials will reach the environment. However, the environmental hazard they pose is poorly understood. The use of *in vitro* models can contribute to increased understanding of molecular and cellular mechanisms in a high-throughput and cost-effective manner and can address the challenge of the plethora of nanomaterials (NM) continuously produced and released in the environment. Fish cell lines can be versatile tools as non-animal methods (NAMs) to assess the effects of NM in the aquatic environment. The aim of this study was to elucidate the behavior, biocompatibility and effects of the advanced NM MoS_2 and Mg-Al-LDH *in vitro* using the zebrafish liver cell line (ZFL) and to evaluate its suitability in nanotoxicology studies.

Methods. Negatively-charged defect-rich, enlarged interlayer MoS_2 nanosheets and positively-charged Mg-Al-LDH layered nanosheets were synthesized. The NM were dispersed in MilliQ water using probe sonication according to established protocols. The characterization of the NM in the stock dispersions and exposure media (ZFL media in the absence of fetal bovine serum) was performed with transmission electron microscopy (TEM) and dynamic light scattering (DLS). The ZFL cell line was used as an *in vitro* model to assess the impact of the NM. The cells were exposed to increasing concentrations of MoS_2 and LDH for 24-48 h. Effects on metabolic activity, membrane integrity, lysosomal integrity and reactive oxygen species (ROS) formation were subsequently assessed. NM interference controls were included to assess their potential interference with the respective assays.

Results. Both material were shown to lack dispersion stability under the exposure conditions over time. A decrease in size was observed during exposure over time resulting, which is probably due to the settling of material which corresponds to a decrease in particle count rates over time. A decrease in metabolic activity and membrane integrity was observed after exposure to the highest concentrations of MoS_2 nanosheets, while no effects were observed for LDH NM. Both MoS_2 and LDH led to an increase in ROS formation which was the most sensitive endpoint in the ZFL cell line.

C*onclusion*. The first results of the study show no adverse effects of the LDH NM despite the high exposure concentrations used, which suggests their suitability as a novel material for environmental applications. In the case of the MoS_2 nanosheets, a decrease in the metabolic activity and membrane integrity was observed, albeit at high concentrations. Ongoing work focuses on NM uptake studies as well as comparing *in vitro* with *in vivo* responses using the zebrafish embryo test.

Effect of contaminants on the transcriptomic response of endothelial cells in low oxygen and high pressure conditions in northern elephant seals, human and sheep.

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Objectives: Large marine mammals play crucial roles as mid or top predators in marine food webs. Top predators' high energy intake is often linked to elevated absorption of contaminants with bioaccumulating and biomagnifying properties, which makes them ideal subject studies. Nowadays, the uncontrolled release of new chemicals into the environment through industrial discharges, improper waste disposal, and accidental spills, as well as the presence of legacy chemicals, poses a serious threat to ecosystems and wildlife. Elephant seals are top predators and expert divers able to survive repeated and extended periods of exposure at high pressure and low-oxygen conditions. To date, the mechanisms behind these adaptations are not fully understood, and only a handful of studies have given mechanistic insights into contaminant response in these marine mammals. To fill in this gap, we developed an *in vitro* method using endothelial cells extracted from the placenta artery of elephant seals, human, and sheep. The goal is to characterize the difference in transcriptional response between diving and non-diving mammal cells exposed to contaminants and/or subjected to high pressure and low-oxygen environments.

Methods: The cells will be expanded and exposed to Mono(2-ethylhexyl) Phthalate (MEHP) or Benzo(a)pyrene (B(a)P) for 48 hours, followed by one hour in high pressure or hypoxic conditions at 1% oxygen. This method will reproduce the incredible dives of those individuals and RNA-sequencing will allow us to study the effect of contaminants on these adaptations. MEHP and B(a)P are both relevant contaminants to study as their occurrence in the tissues of marine mammals has been reported. They are also known endocrine-disrupting compounds so we have reasons to believe that they might alter cells resistance to hypoxia. The effect of high pressure on cells has received limited attention. From an evolutionary perspective, this investigation has the potential to initiate additional studies and the development of new methodologies.

This project is part of the Marma-detox Project, funded by the Research Council of Norway, project no. 334739, 2023-2027.

Killer whale (*Orcinus orca*) fibroblast; optimizing culture conditions and unveiling toxicological insights of persistent organic pollutants (POPs)

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Objectives: Killer whale is a cetacean with a worldwide distribution. As a top predator, the killer whale has been found to have over 140 species as prey high up in the marine food web. These whales are therefore exposed to pollutants that have both bioaccumulative and biomagnifying properties. To assess the effects of pollutants on killer whales, fibroblast cultures from skin biopsies will be established and used in *in vitro* exposure experiments.

Methods: Optimization of fibroblast growth was done in three steps. The first step tested the use of different concentrations of human fibroblast growth factor - basic (bFGF), to increase cell proliferation, alongside the antioxidant N-acetyl cysteine (NAC), to mitigate production of reactive oxygen species (ROS). The second step involved different coatings of culture plates with fibronectin (0,02mg/ml), gelatin (0,1%), collagen (0,01%) and laminin (0,01%) including a control, no coating, and cell + plates. The third step explored the impact of hypoxia (5 % O_2) and normoxia, both with and without NAC. All three steps were evaluated by cell counting and lactic acid dehydrogenase (LDH) cytotoxicity assay. Step one also included a CM-H_2DCFDA as an indicator for ROS in cells. A glutathione assay kit was used to monitor total glutathione ratio changes in the third optimization step.

Results and conclusion: The cells will be exposed to p,p'-dichlorodiphenyldichloroethylene (p,p-DDE), a metabolite of DDT, and PCB118 which both are found in high concentrations in killer whales. Previous studies have shown p,p-DDE to target the receptor thyroid hormone receptor (THRB) and the protein extracellular signal-regulated kinase (ERK). While PCB 118 have been shown to affect the aryl hydrocarbon receptor (AHR) and its downstream target gene cytochrome P450 1A (CYP1A). Mono(2-ethylhexyl) phthalate (MEHP), a metabolite of DEHP, will also be assess concerning its effect on peroxisome proliferator-activated receptor gamma (PPARG) and its target gene adiponectin (ADIPOQ). Lastly, the effect of perflourooctane sulfonic acid (PFOS) on PPAR-alpha and its target gene CYP4A will be assessed. This work will contribute to elucidating how well killer whales handle their loading of environmental pollution.

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Optimization, exposure and reprogramming of fin whale cells

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Objectives

This MSc project is a part of the Marma-detox project, which tests effects of contaminants on molecular targets in cells from marine mammals by in vitro and in silico methods. This is a work in progress, with focus on the fin whale. The project is divided into three parts: optimization of growth conditions of fibroblasts, exposure of the fibroblasts to environmental pollutants and reprogramming of fibroblast cells to mesenchymal stem cells (MSCs).

Methods

The optimization experiment is divided into three parts: 1. Providing the cells with combinations of different concentrations of basic fibroblast growth factor (bFGF), and N-acetylcystein (NAC). 2. Growing the cells in culture dishes with different types of coating: +wells, laminin, collagen, gelatin and fibronectin. 3. Growing the cells in hypoxia chamber, which would provide them with an environment of 5% oxygen and is closer to in vivo environment.

The exposure experiment will consist of treating the cells with the pollutants dichlorodiphenyldichloroethylene (p,pDDE), 2,3',4,4',5-pentachlorobiphenyl (PCB118), mono-2-ethylhexyl phthalate (MEHP) and hexachlorobenzene (HCB). qPCR will be used to test their effects on their different target receptors: THRB, ERK, AhR, CYP1A, PPARG, and ADIPOQ. In addition, measuring DNA breaks will preferably be tested on the cells treated with HCB, as this is a known consequence.

The reprogramming of the fibroblasts will be done by PCR-amplifying and introducing four of the so-called Yamanaka factors: OCT4, KLF4, SOX2 and GLIS1.

Results

The current results shows that a concentration of 5mM bFGF gave a higher number and better viability of cells. Concentrations of NAC that of 2,5mM gave a higher cell number and viability, while concentrations above that gave a lower number and higher mortality of the cells, with 10mM giving only about a tenth of the cell number as the control and a mortality rate of more than 60%. From the coating experiment, laminin, collagen and gelatine had a slight increase in cell number, while fibronectin had a decrease. The +wells, on the other hand, gave an almost double cell number as the control.

Conclusion

The NAC and bFGF concentrations were used in the subsequent experiments, which resulted in a considerable increase in cell growth and confluency time. After the experiment, pH change resulting from NAC was neutralized. Redoing the experiment with this in mind could be beneficial, in addition to testing higher concentrations of bFGF.

The Marma-detox Project is funded by the Research Council of Norway, project no. 334739, 2023-2027

ERT-presentation

The total antipsychotic burden in patients using Olanzapine in combination with one or more antipsychotics in different age- and sex groups

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Objectives

Even though guidelines recommend antipsychotic monotherapy, several patients use a combination of multiple antipsychotic drugs. Several studies have shown that advancing age, as well as being female, tends to result in higher concentrations of antipsychotic drugs despite low doses. The aim of this study was to investigate the impact of age and gender on overall antipsychotic burden in patients using olanzapine in combination with one or more antipsychotics.

Methods

The retrospective study was based on data from the therapeutic drug monitoring (TDM) service at Center for Psychopharmacology, Diakonhjemmet Hospital of patients using olanzapine alone or in combination with one or more other antipsychotics. Information about age, gender, daily dose and the timing of blood sample collection in relation to dose intake were available from the requisition forms. The serum samples were excluded if the age was under 18 years, the daily dose of olanzapine was below 5 mg, or in the form of depot formulation, serum concentration outside quantification limits, and the time from sample collection was beyond 12-24 hours after the last dose of the medication. For each patient, the total olanzapine dose equivalents and concentration equivalents were calculated using conversion factors. Females and males were grouped into 3 age groups (18-49, 50-74, 75+).

Results

In total, 19148 patients had a serum concentration measurement of olanzapine between January 2010 and September 2023. After exclusion, the study population consisted of 9705 (5208 male, 4497 female) patients. The mean age of the study population was 48 years, and the mean daily dose was 13 mg. Among the patients 14%, used a combination of antipsychotics. The total antipsychotic burden was highest among young males with 17,24 mg olanzapine dose equivalents and 120,78 nmol/L concentration equivalents, and lowest among those over 75 with 8,86 mg dose equivalents and 97,12 nmol/L concentration equivalents for males and 8,50 mg dose equivalents and 105,82 nmol/L concentration equivalents for females. With increasing age, it is observed that olanzapine dose equivalents decrease by 51%, while concentration equivalents only decrease by a corresponding 20%. Analyses to examine the results further are still ongoing.

Conclusion

The preliminary findings indicate that older (75+), female olanzapine-treated patients have a high antipsychotic burden, measured as serum concentrations, despite being prescribed lower doses of antipsychotics.

Pharmacokinetic models in personalized dosing of vancomycin

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Objectives

To evaluate the current routines for trough-based vancomycin dosing in Helse Vest, and to identify a pharmacokinetic model for individual, area under the curve (AUC)-guided vancomycin dosing for the patient population.

Methods

In a prospective cohort study, we collected data in patients treated with vancomycin from three different hospital departments in Helse Vest. The data consists of a set of variables, including doses and serum concentrations of vancomycin, creatinine, sex, age and BMI.

The variables were used to compare a selection of available pharmacokinetic models to find the one that best describes our population. PrecisePK® is a commercial software that can be used for individual AUC-guided vancomycin dosing, and the pharmacokinetic models that are utilized in PrecisePK® were compared with other published models using the non-linear mixed effects modeling software Monolix®.

The log-likelihood was used to assess the goodness of fit of the models to our population data, and Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used to compare the different models.

Results

Data have so far been collected from 40 of the patients included in the prospective study (17 women and 23 men) with a total of 146 vancomycin serum concentrations. The inclusion is ongoing, and the present dosing routines will be evaluated when the data collection is completed.

Initial modeling has been performed in Monolix®, using dose and serum concentrations of vancomycin as variables. Comparing a one-compartment model and a two-compartment model for vancomycin gives lower AIC and BIC-values for the one-compartment model, which means that this model is the best fit for our data at this point in our data handling.

Conclusion

Preliminary results show that a one-compartment model might be adequate to describe the pharmacokinetic of vancomycin in our population. More variables will be added to the model to confirm this.

Microbiome-derived reactivation of mycophenolic acid in healthy individuals

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Objectives
Studies have shown that the gut microbiome may be a significant source of the variation seen in mycophenolic acid (MPA) pharmacokinetics. In the liver, MPA is glucuronidated to the inactive main metabolite mycophenolic acid glucuronide (MPAG) by uridine diphosphate-glucuronosyltransferase enzymes. MPAG is then excreted to the gastrointestinal tract through the biliary ducts, where bacterial β-glucuronidase enzymes reactivate MPA, which then can be reabsorbed. This enterohepatic recirculation results in an increased systemic exposure of MPA, which is often shown as a secondary peak in the time-concentration curve. We have previously determined the microbiome-derived reactivation of MPA in renal transplant recipients. The aim of this project was to determine the microbiome-derived reactivation of MPA in fecal samples from healthy individuals and to compare these with the MPA reactivation rates in renal transplant recipients.
Methods
Fecal samples obtained from a previously conducted clinical trial (IntraCYP) were utilized in this project. To isolate proteins and enzymes from the fecal samples, concentrated lysates were prepared using bead beating and sonication as cell lysis techniques. The total protein concentration in the lysates was determined using a Bradford assay, and then diluted to reach a total protein concentration of 0.2 mg/mL. Next, the reactivation rate in the protein lysates was determined. MPAG (100 mg/L) was added to the lysates and then incubated in a water bath at 37°C. Samples for determination of MPA and MPAG concentrations were obtained at the time points: 0 min, 15 min, 30 min, 1 hr, 1.5 hr, and 2 hr. UHPLC-MS/MS was used to determine concentrations of MPA and MPAG in the lysates. The reactivation rate of MPA was determined using a linear regression function of cumulative MPA concentrations as a function of time, with the slope representing the reactivation rate.
Results and conclusions
So far, lysates have been prepared for a total of 44 fecal samples from 16 different healthy individuals included in the IntraCYP study. In these lysates, the reactivation rate of MPA will be determined and further compared to the previously determined reactivation rates in renal transplant recipients. Preliminary results will be presented at the meeting.

Contribution of miRNA content in NK cell-derived extracellular vesicles to apoptosis of cancer cells

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NK cells release extracellular vesicles (EVs) in response to cytokine stimulation or receptor engagement. These vesicles contain cytolytic proteins, and can induce apoptosis of a wide range of cancer cells. The EV secretome is heterogeneous, and we previously showed that NK cells release distinct vesicles originating from the plasma membrane or from intracellular sources. Only the intracellularly-derived EVs mediate apoptosis of cancer cells. To further understand how these vesicles target and kill cancer cells, we profiled the miRNA expression in EV subpopulations derived from NK cells via the NanoString hybridization platform and quantitative PCR. We found that there was an enrichment of miR-19b-3p, miR-20a/20b-5p, miR-23a-5p, miR-29b-3p, miR-142-3p, miR-181a-5p, miR-630, and miR-1246 in internally-derived cytotoxic EVs. Target prediction analysis suggested that the miRNAs converge on genes related to apoptosis, and we show that the EVs reduced expression levels of the anti-apoptotic genes *BCL2*, and *RICTOR* in the HCT-116 colon cancer cell line. We further tested whether individual miRNA mimics could replicate induction of HCT-116 apoptosis, and via a screen of 9 miRNAs detected in NK-EVs, show that miR-19, miR-142 and miR-29b induced a dose-dependent increase in apoptosis and reductions of *BCL2* gene expression, indicating that these miRNAs may contribute to the apoptotic effect of the NK-EVs.

Dissecting intracellular sources and release mechanisms of extracellular vesicles from NK cells

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NK cells release extracellular vesicles (EVs) in response to cytokine stimulation or receptor engagement. Our aim is to utilize NK-EVs for cancer therapy, and with this project we aim to identify conditions that yield release of cytolytic EVs, and also understand from which intracellular compartments they are released. While multivesicular bodies are the main source of vesicles, we find that also secretory cytolytic granules contain intraluminal vesicles. How the release of intraluminal vesicles is controlled is currently unknown. To understand this, we compared the vesicular secretome of NK cells stimulated through either the activating receptor CD16 or via cytokine stimulation. Vesicles originating from secretory granules uniquely contain the tetraspanin NKG7, and can thus be used as marker for these vesicles. Our preliminary experiments indicate that vesicles released in response to CD16 stimulation may be more cytotoxic than vesicles released upon cytokine stimulation, which indicates that vesicles contained in granules may control induction of tumor cell apoptosis. We are further testing how blockade of Rab proteins and other proteins involved in the exocytosis machinery may govern differential release of the vesicles. Ultimately, this information will be necessary when designing protocols for production of therapeutic NK-EVs.