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12th Swiss Pharma Science Day 2019

Wednesday August 28, 2019

University of Bern, Langhans Auditorium, Pathology Building and House of the University of Bern

«DIABETES»

Intention

The SWISS PHARMA SCIENCE DAY (SPhSD) is an annual event of the Swiss Academy of Pharmaceutical Sciences (SAPhS, www.saphw.ch). The 1st SPhSD was held on October 9, 2008, at the University of Bern. For congress reports 2008-2018 including all lecture and poster abstracts see www.saphw.ch. The SPhSD offers a platform to present, in form of a poster session, the latest research results of Master and PhD students, as well as Post-Docs of all the three Swiss Academic Institutions for Pharmaceutical Sciences, i.e. ETH Zurich, School of Pharmaceutical Sciences of the Universities of Geneva and Lausanne (EPGL) in Geneva and the University of Basel. Master students of the Universities of Applied Sciences, i.e. FHNW (School of Life Sciences, Muttenz) and ZHAW (Life Sciences and Facility Management, Institute of Biotechnology, Wädenswil) are also invited to participate in this event.

The poster session is embedded in a series of lectures given by invited distinguished scientists representing the broad field of pharmaceutical sciences, such as Pharmaceutical Biology, Biotechnology, Technology, Chemistry, Analytics, Engineering, Pharmacology, or Molecular Biology.

One of the primary goals of the SPhSD is to further stimulate professional and social contacts between the students still undergoing training and Alumni, having already a position in industry, hospital, public health administration or public pharmacy. Thus, cooperation and networking between the different institutions in academia and industry and the different fields of pharmaceutical sciences is being promoted.

Last but not least, the SPhSD represents an ideal platform to meet young engineers and scientists, who may be recruited for a position in the academia, hospital, industry, public health administration or public pharmacy.

Organizing Committee:

Prof. Dr. Rudolf Brenneisen, SAPhS, Secretary General, Bern

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Prof. Dr. Gerrit Borchard, SAPhS, President

School of Pharmaceutical Sciences EPGL, University of Geneva

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Program

«Diabetes»

9:15-9:45 h	**Registration**
9:45-10:00 h	**Addresses of Welcome** - Prof. Dr. Gerrit Borchard University of Geneva, President SAPhS - PD Dr. Verena Schröder University of Bern, Dept. BioMedical Research - Prof. Dr. Rudolf Brenneisen Secretary General SAPhS - Prof. Dr. Hans Leuenberger University of Florida, Former President SGPhW
	Morning Session Chair: Prof. Dr. Hanns-Christian Mahler, Lonza AG Basel
10:00-10:30 h	**Lecture 1: Keynote** Prof. Dr. Pierre Maechler University of Geneva Medical School (CMU): **«Limiting Insulin Secretion as an Anti-Obesity Therapeutic Strategy»**
10:30-11:00 h	**Coffee**
11:00-11:30 h	**Lecture 2: Pharmacology** Dr. Yossi Tam The Hebrew University of Jerusalem, Israel: **«Diabesity-Induced Chronic Kidney Disease: When Kidneys Get the Munchies»**

Program (cont.)

11:30-12:00 h	**Discussion Lecture 1 and 2**
12:00-14:00 h	**Lunch and Poster Session**
	Afternoon Session **Chair: Prof. Dr. Jörg Huwyler, University of Basel**
14:00-15:00 h	**Short Oral Presentations – Selected Abstracts**
14:00-14:10	M. Faria Freitas, A.A. Leslie Gunatilaka, M. Cuendet EPGL, University of Geneva: **«Withanolides Inhibit Cell Proliferation in a Multiple Myeloma 3D Co-Culture Model Enriched with Cancer Stem Cells»**
14:10-14:20	C. Lamers, B. Wagner, P. Gros, J. D. Lambris, D. Ricklin Dept. Pharm. Sciences, University of Basel: **«Structure-Activity Study and Molecular Insights in the Mode of Action of Complement C3 Inhibitor Cp40»**
14:20-14:30	L. D. Simmler, R. Van Zessen, L. C. Hadjas, Ch. Lüscher EPGL, University of Geneva: **«Acute Reinforcement, But Little Adaptive Behavior With Ketamine»**
14:30-14:40	F. Borgna, C.A. Umbricht, L. Deberle, R. Schibli, C. Müller Center for Radiopharm. Sci., Paul Scherrer Institute Villigen: **«Combination of Albumin-Binding 177Lu-PSMA-ALB-56 and Fast-Cleared PSMA-Inhibitors: Optimization of the Pharmacokinetics»**
14:40-14:50	V. Patrulea, O. Jordan, B.H. Gan, E. Sublet, K. Perron, J.-L. Reymond, G. Borchard EPGL, University of Geneva: **«Highly Engineered Antibacterial Peptide Dendrimers Coupled to Chitosan Derivatives to Efficiently Eradicate Pseudomonas aeruginosa»**
14:50-15:00	I. Younes, V. Patruela, V. Frachet, A. Boumendjel, O. Jordan, G. Borchard EPGL, University of Geneva: **«A New Chalcone Derivative with High Pro-Apoptotic Effect on Cisplatin-Resistant Bladder Carcinoma»**

Program (cont.)

	Afternoon Session (cont.) **Chair: Prof. Dr. Jörg Huwyler, University of Basel**
15:00-15:30 h	**Lecture 3: Nanotechnology** **Prof. Dr. Randall J. Mrsny** **University of Bath, Bath U.K.:** **«A Carrier-Mediated Approach for the Oral Delivery of Protein Therapeutics»**
15:30-16:00 h	**Lecture 4: Prenatal Pharmacology** **PD Dr. med. Marc Baumann** **University Hospital-Inselspital Bern:** **«Diabetes in Pregnancy and Fetal Programming – From Basic Science to Clinics»**
16:00-16:30 h	**Discussion Lecture 3 and 4**
16:30-16:45 h	**Coffee**
16:45-17:15 h	**Award Ceremony**
17:30-18:30 h	**Apéro at the House of the University of Bern**

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Lectures

L-1

Pierre Maechler, Prof. Dr., University of Geneva Medical School (CMU):

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CV:

Education:

1982: Baccalaureate in Science (maturité fédérale type C).
1982-1986: Bachelor, University of Geneva, Faculty of Sciences, section of Biology.
1985-1986: Master degree: laboratory of Prof. M. Schapira (Faculty of Medicine) under the supervision of Prof. U.K. Laemmli (Faculty of Sciences): «Activation of human neutrophils by plasma kallikrein».
1990-1993: Ph.D. thesis at Symphar-ILEX Pharmaceutical Research (Versoix/San Antonio) delivered by the University of Geneva: «Study on the mechanisms leading to diabetes-associated hypercholesterolemia in rats with experimental diabetes».
2002: Privat Docent thesis (lecture on September 23rd): «Mitochondria as the conductor of metabolic signals for insulin exocytosis in pancreatic ß-cells».

Employment:

1987-1995: Research Scientist at Symphar-ILEX Pharmaceutical Research Laboratory, Versoix/San Antonio.
1995-2000: Senior Scientist (Maître-Assistant) at the Division of Clinical Biochemistry, HUG, Geneva.
2001-2006: Head of a junior group, fellow of the Dr Max Cloetta foundation: Maître d'Enseignement et de Recherche Suppléant (MERs) at the Dept. of Cell Physiology and Metabolism, CMU, Geneva.
2006-2011: Associate Professor (Professeur Adjoint) at the Dept. of Cell Physiology and Metabolism, University Medical Center, Geneva.
2011-present: Full Professor at the Dept. of Cell Physiology and Metabolism,CMU, Geneva.

Active memberships in scientific societies:

- Société Académique de Genève
- Swiss Endocrine & Diabetes Society (president of the Research Commission: 2006 - 2011)
- Life Science Switzerland (LS2), former USGEB - Swiss Society for Molecular and Cellular Biosciences
- American Society for Biochemistry and Molecular Biology (ASBMB)
- European Association for the Study of Diabetes (EASD)
- Faculty Diabetes Center (University of Geneva)
- EASD Islet Study Group (vice-chairman 2014 – 2017)
- Centre Européen du Diabète (Strasbourg): Scientific board (2012 – present)
- Hjelt Diabetes Foundation: Board member (2018 – present)

Funded projects over the last three years:

- Sinergia – SNF (coordinator): *«Novel avenues in mitochondrial function and diabetes»*
- CTI (principal investigator): *«Discovery and development of novel drug candidates for the treatment of diabetes»*
- Fondation Romande pour la Recherche sur le Diabète: *«Implication of GPR40 in the glucolipotoxicity response of insulin-secreting cells»*
- Swiss National Science Foundation: *«Glutamate pathways and metabolic stresses in energy homeostasis»*

Lecture Abstract:

«Limiting Insulin Secretion as an Anti-Obesity Therapeutic Strategy»

In obese subjects, the pre-diabetic state is characterized by hyperinsulinemia secondary to insulin resistance [1], which may lead to diabetes in case of subsequent ß-cell failure [2]. However, ß-cell-targeted genetic intervention in mice, limiting ß-cell function, may protect against obesity. Glucose-stimulated insulin secretion from the pancreatic ß-cell comprises two modalities, the obligatory calcium-mediated signalling and the more long-lasting amplifying pathway. The latter depends on the cataplerotic activity of the mitochondrial enzyme glutamate dehydrogenase (GDH) [3]. GDH catalyses the following reaction: α-ketoglutarate + NH3 + NADH ↔ glutamate + NAD+. Moreover, GDH is allosterically regulated by leucine as well as pyridine, adenine and guanine nucleotides; being inhibited by GTP and activated by ADP. Regarding gain of function mutations, the GDH-S445L mutation confers hyperactivity to the enzyme due to higher sensitivity to ADP allosteric activation. This renders ß-cells responsive to amino acid stimulation, explaining protein-induced hypoglycemia [4]. Regarding loss of GDH function, mice lacking GDH in ß-cells provide evidence that the absence of GDH-dependent amplifying pathway preserves a lean phenotype by limiting insulin release to levels preventing excessive fat storage and the accompanying insulin resistance [5]. At the cellular level, in both rodent and human islets, inhibition of GDH by the polyphenol EGCG reproduces the effects of GDH knockout in the ß-cell [6]. Clinical data have shown that pharmacological inhibition of insulin secretion in obese subjects can promote weight loss [7, 8], although drugs used in such studies are associated with undesired side effects, such as hyperglycemia. Fine tuning of GDH and insulin secretion is a major challenge for the management of both obesity and diabetes.

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[1] Kasuga M. Insulin resistance and pancreatic beta cell failure. J Clin Invest 2006;116: 1756-1760

[2] Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest 2006;116:1802-1812

[3] Vetterli L, Carobbio S, Pournourmohammadi S, Martin-Del-Rio R, Skytt DM, Waagepetersen HS, Tamarit-Rodriguez J, Maechler P. Delineation of glutamate pathways and secretory responses in pancreatic islets with beta-cell specific abrogation of the glutamate dehydrogenase. Mol Biol Cell 2012; 23: 3851-3862

[4] Grimaldi M, Karaca M, Latini L, Brioudes E, Schalch T, Maechler P. Identification of the molecular dysfunction caused by glutamate dehydrogenase S445L mutation responsible for hyperinsulinism/hyperammonemia. Human molecular genetics 2017; 26: 3453-3465

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[8] van Boekel G, Loves S, van Sorge A, Ruinemans-Koerts J, Rijnders T, de Boer H. Weight loss in obese men by caloric restriction and high-dose diazoxide-mediated insulin suppression. Diabetes, obesity & metabolism 2008; 10: 1195-1203

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CV:

Dr. Yossi Tam received his B.Med.Sc., M.Sc., Ph.D. (Magna Cum Laude) and D.M.D. from the Hebrew University of Jerusalem. He did his postdoctoral training at the National Institutes of Health (NIH), and in 2011, he became a Staff Scientist at the NIH. In June 2014, Dr. Tam moved to the Hebrew University, where he won major national and international grants (ISF, GIF, EFSD, Abish-Frankel, FPWR, TOS, and the prestigious European Research Council (ERC) Starting Grant). He authored over 40 peer-reviewed papers in leading journals (such as, Nature Medicine, Cell Metabolism, JCI, PNAS, Hepatology, Gastroenterology, Molecular Metabolism, to name a few), and two book chapters.

Dr. Tam's research projects over the past seventeen years has crossed subjects, disciplines and methodologies, yet the main research interests remain focused on the different pathophysiological aspects of the endocannabinoid (eCB) system. Having a clinical background with basic science training, he has always been interested in how science can directly improve people's everyday lives. Thus, he has strived unceasingly to integrate his clinical curiosity and experimental knowledge, in order to deepen the understanding of clinically relevant research questions.

Dr. Tam is the head of the Obesity and Metabolism Laboratory at the Institute for Drug Research, where he focus on targeting the eCB system for Obesity, Diabetes and the metabolic syndrome. He also serves as the Director of the Hebrew University's Multidisciplinary Center on Cannabinoid Research and a Scientific Advisory Board Member of several biotech companies, which develop a portfolio of non-psychoactive cannabinoid and cannabinoid-modulating medicines for unmet market needs.

Lecture Abstract:

«Diabesity-Induced Chronic Kidney Disease: When Kidneys Get the Munchies»

Diabetes and obesity (now termed diabesity), chronic diseases that are now reaching epidemic proportions, have been described as catalysts for many conditions, most notably, cardiovascular disease, liver disease, and chronic kidney disease (CKD). The latter is manifested by hemodynamic and morphological changes in the kidney, which together with renal inflammation and oxidative stress, may lead to reduced renal function and ultimately, to glomerulosclerosis and tubulointerstitial fibrosis. Although multiple metabolic factors have been proposed to contribute to diabesity-induced CKD, the underlying signaling mechanisms are not completely understood.
The recreational, psychoactive, and medicinal effects of marijuana, many of which have important therapeutic potential, have been recognized for thousands of years. Yet, it is only in the last several decades that our understanding of these effects has grown, following some landmark discoveries in the field of cannabinoid research. Endocannabinoids (eCBs) are endogenous lipid ligands that bind to cannabinoid receptors (CB1 and CB2) that also mediate the effects of marijuana. The eCB system is present in both the central nervous system and peripheral organs including the kidney. Accumulating evidence has described the role of the eCB system in various renal pathologies. CB1 receptors are

expressed in podocytes, mesangial cells, and particularly in renal proximal tubular cells (RPTCs). Their blockade with CB1 receptor antagonists improves renal function and reduces albuminuria and glomerular lesions in obese and diabetic mouse models. However, most of these studies failed to determine whether the eCB system plays a role in diabesity-associated renal pathologies. And if it does, is it mediated centrally, peripherally, or via a specific cell type within the kidney?

Our results, which will be presented during the 12th Swiss Pharma Science Day (SPhSD), will describe novel cellular mechanisms by which the CB1 receptor regulates glucose and fat utilization as well as mitochondrial shape and function in RPTCs. Our findings indicate that diabetes-induced upregulation in renal glucose absorption via the facilitative glucose transporter 2 (GLUT2) is mitigated by pharmacological blockade or genetic ablation of the CB1 receptor in RPTCs, by inducing changes in Ca^{2+} influx and PKC-β1 expression to reduce glucose reabsorption and prevent the development of CKD [1]. In parallel, lipid accumulation and reduced fatty acid β-oxidation in RPTCs, associated with obesity-induced renal abnormalities, are governed by a CB1 receptor-coupled $G\alpha_{i/o}$-PKA axis, which mediates the downstream activation of the LKB1/AMPK/ACC signaling pathway [2]. The direct role of the CB1 receptor in renal lipotoxicity and kidney damage is also mediated, in part, by inducing mitochondrial fragmentation via changing the phosphorylation levels of the canonical fission protein dynamin-related protein 1. This, in turn, is associated with mitochondrial dysfunction in RPTCs [3].

Since the therapeutic potential of globally acting CB1 receptor antagonists in diabesity is limited due to their neuropsychiatric adverse effects, our recent findings support the pre-clinical development and clinical testing of peripherally restricted CB1 receptor antagonists in treating renal diseases.

References:

[1] J Am Soc Nephrol. 2018; 29:434-448
[2] J Am Soc Nephrol. 2017; 28:3518-3532
[3] Diabetes Obes Metab. 2019; 21:146-159

L-3

Prof. Dr. Randall J. Mrsny, University of Bath, Bath U.K.

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CV:

Randy Mrsny obtained a B.S. in Biochemistry and Biophysics at the University of California at Davis, a Ph.D. in Anatomy and Cell Biology at the U.C. Davis School of Medicine and spent four years as an NIH Postdoctoral Fellow in Membrane Biophysics in the Institute of Molecular Biology at the University of Oregon. He ran the Peptide Biology Group at ALZA Corp and then the drug delivery/biology group at Genentech. Randy currently holds a Professor's chair of Epithelial Cell Biology at the University of Bath in the Department of Pharmacy and Pharmacology where he studies biological principles associated with normal epithelia cell function and how these are affected in disease states. He is also the CSO of Applied Molecular Transport, which utilizes endogenous cell pathways for improved oral drug delivery. Randy has been elected president of the Controlled Release Society and to co-organize a Gordon Conference on Drug Delivery. He was recently selected to the top 100 Medicine Maker's Power List for people involved in drug development and manufacturing.

Lecture abstract:

«A Carrier-Mediated Approach for the Oral Delivery of Protein Therapeutics»

Bacteria use a variety of protein-based virulence factors to facilitate/stabilize infections at mucosal surfaces, such as those found in the gut and lung. These virulence factors commonly have a cytotoxic element that functions to damage the barrier properties the polarized epithelium at these mucosae to allow pathogen access the host. We have, however, demonstrated a novel mechanism of virulence for the cholix protein secreted from non-pandemic strains of Vibrio cholerae. Cholix hijacks a transcytosis trafficking pathway to move quickly across the epithelium of the intestine, allowing it to selectively reach and intoxicate cells within the lamina propria. We have focused on identifying hallmarks of the pathway hijacked by cholix that are used in the transcytosis pathway that ensure the apical to basal active transport of a biologically-active, fully-intact virulence protein. This pathway appears to represent a previously unappreciated mechanism that bacterial infections can use at mucosal surfaces to facilitate/stabilize infections. We have identified the domain of cholix that harbors this transcytosis function and used it to orally delivery a therapeutic protein, showing that the pathway and properties of cholix have now been sufficiently characterized to allow manipulation for pharmaceutical applications. We believe our work opens a new door in the area of understanding how agents secreted by microbiome elements at mucosal surfaces might clandestinely manipulate events in the underlying submucosa and demonstrate an application of this novel biology.

PD Dr. med. Marc Baumann,University Hospital-Inselspital Bern

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Marc Ulrich Baumann
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Education and professional experience:

1990	Zurich	Highschool, Kantonsschule Limmattal (Urdorf)
1990-1992	Geneva	Study of Medicine at the University of Geneva, 2nd propedeutical exam
1992-1996	Zurich	Study of Medicine at the University of Zurich
1997	Geneva	US Federal exam of Medicine (USMLE, Educational Comission for Foreign Medical Graduates)
1998-1999	Samedan	Resident, Surgery, Hospital Oberengadin, Samedan
1999	Zurich	Thesis «E-selectin upregulation following spinal cord injury in mice»; Brain Research Institute of Zurich, Prof. M. Schwab
1999-2019	Bern	Residency, Gyn. and Obstetrics, Women's Health Hospital, Inselspital, Bern
2000-2002	Newark	Postdoctoral research fellowship, Perinatal Biology Group, Dep. of Obstetrics Gynecology and Women's Health, UMD - New Jersey Medical School, USA
2010	Bern	Federal Exam for Gynecology and Obstetrics
2010-2015	Bern	Prinicipal Investigator «Project # 9: Placental transport systems and their impact on fetal programming» i.R. «SNF-NCCR TransCure - From transport physiology to identification of therapeutic targets»
2011-2019	Bern	Fellowship, Gyn. and Obstetrics, Sub-speciality fetomaternal medicine, Women's Health Hospital, Inselspital Bern
2015	Bern	Venia docendi (Privatdozent) at the University of Bern

Memberships/activities:

2000	International Federation of Placenta Associations (IFPA)
2002	International Society of Ultrasound in Obstetrics and Gynecology (ISUOG)
2009	European Placental Group (EPG) planning committee member
2010	Member of the Swiss Academy of Feto-maternal Medicine
2013	President, Swiss Branch of the International Society of the Study of Hypertension in Pregnancy (ISSHP)
2015	Member SGGG / gynécologie Suisse
2016	Member SRI, Society for Reproductive Investigation
2019	Military: Lieutenant colonel, Chief SanDienst, Heeresstab

Lecture abstract:

«Diabetes in Pregnancy and Fetal Programming – From Basic Science to Clinics»

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first detection during pregnancy. Affecting up to 10% of all pregnancies worldwide, GDM has an important impact on society. GDM substantially contributes to pregnancy complications and, in turn, to both maternal and neonatal morbidity and mortality. Furthermore, following pregnancies affected by GDM, the risk for complications later in life is increased for both the mother and her child; a concept which is known as

«fetal programming». The intrauterine exposure of the fetus to a variety of substances and molecules has an impact on the development of metabolic and cardiovascular diseases. Uric acid metabolism, specifically hyperuricemia, is believed to be involved in the pathogenesis of GDM. Recently it has been recognized that regulation of uric acid serum levels depends largely on the uric acid transporter GLUT9 (SLC2A9) which is responsible for uric acid reabsorption in the kidney. GLUT9 is also present in various other organs such as liver, intestine and placenta, although its role in these organs is still poorly understood. The profound knowledge of the regulation of the placental uric acid transport system and its transporter GLUT9 will be the key prerequisite for the development of novel therapeutic strategies for the treatment and prevention of GDM and its longterm consequences on affected mothers and children.

Posters

P-1

Additives in Polyelectrolyte Matrices – A Synchrotron Study

F. Ditzinger[1,2], C. Dejoie[3], D. Schumacher[2], M. Kuentz[1]

[1] *University of Applied Sciences and Arts Northwestern Switzerland, Institute of Pharma Technology, 4132 Muttenz, Switzerland*
[2] *University of Basel, Department of Pharmaceutical Sciences, 4056 Basel, Switzerland*
[3] *European Synchrotron Radiation Facility, 38000 Grenoble, France*

Introduction: Formulating poorly water-soluble drugs as amorphous solid dispersions (ASD) has been a well-established process in the pharmaceutical industry. A manufacturing process for ASDs is hot melt extrusion (HME), in which polymeric compounds are formulated with drug substances through a melting and a subsequent resolidification step. Some compounds such as polyelectrolytes are of particular interest for the formulation of poorly water-soluble APIs but not applicable for HME. To enable the processing of these substances, the polyelectrolyte sodium carboxymethyl cellulose (NaCMC) was combined with urea, meglumine, tromethamine (TRIS), separately. In a combination of a solvent evaporation and an extrusion step the formuations were manufactured. These formulations were evaluated analytically by differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD) and Attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR). Additionally, to achieve sufficient data over the full range of Q values X-ray patterns were recorded at the European Synchrotron Radiation facility.
Methods: The solvent evaporation process was performed on a laboratory rotary evaporator from Büchi. The subsequent extrusion was performed on a co-rotating twin-screw extruder ZE9 ECO from Three-Tec. The 3 heating zones were set to 130 °C. The recorded 2θ range reached from 5° to 40°. The DSC experiments were performed on a DSC 3 by Mettler Toledo with a heating ramp from 0° to 140 °C (10°C/min). The ATR-FTIR spectra were recorded on a Cary 680 Series FTIR at a scanning range of 4000 to 600 cm^{-1} with 42 scans and a resolution of 4 cm^{-1}. The high-energy synchrotron PXRD measurements of the hot melt extrudates were performed at the European Synchrotron Radiation Facility.
Results: The initial evaluation of the maximum amount of amorphous additive in the poly-electrolyte showed significant differences between the substances. While meglumine was extrudable and amorphous up to 50 % (*w/w*), urea and TRIS could only be used up to 20 % (*w/w*) and 25 % (*w/w*), respectively due to residual crystallinity and recrystallization. This was also reflected in the T_gs of the 3 formulations. For meglumine and urea a T_g was measurable already after the evaporation, whereas amorphous TRIS was only detectable after the additional extrusion. These results were confirmed by synchrotron PXRD.
Conclusions: The use of small molecular additives to enable the extrusion of polyelectrolytes has been proven to be beneficial. The solvent evaporation step represents a necessary step to establish pre-mixture. Moreover, it could be demonstrated that differences in the amorphous additive influence the extrusion behavior of NaCMC. The significance of this study is the extrusion of a polyelectrolyte, which is new to research field of HME and enables further studies to establish highly swellable polyelectrolytes in HME for the solubility enhancement of poorly water-soluble drugs.

Keywords: amorphous solid dispersions, hot melt extrusion, polyelectrolyte matrix, sodium carboxymethyl cellulose

FUNDING: This project has received funding from the European Union's Horizon 2020 Research and Innovation Program under grant agreement No 674909.

P-2

Withanolides Inhibit Cell Proliferation in a Multiple Myeloma 3D Co-Culture Model Enriched with Cancer Stem Cells

M. Faria Freitas[1], A.A. Leslie Gunatilaka[2], M. Cuendet[1]

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Introduction: Multiple myeloma (MM) is a blood disease characterized by the clonal proliferation of malignant plasma cells in the bone marrow. Despite not having a strong incidence, this type of cancer is associated with a high rate of relapse and resistance to conventional therapies. For that reason, there is an urge for new strategies that may include novel treatments or combinations. In order to evaluate the effect of potential treatments, a model that closely represents the disease should be used. Therefore, a 3D co-culture model including cancer stem cells (CSCs), malignant plasma cells and cells from the *in vivo* microenvironment was used. This model not only represents most of the tumor complexity but also includes the interactions with the microenvironment, here represented by the inclusion of mesenchymal stem cells (MSCs). Besides, the addition of CSCs mimics the aggressiveness displayed by *in vivo* refractory tumors.
Aims: The effect on cell viability of 63 withanolides was first assessed in RPMI 8226 cells and MM-CSCs. Compounds with the best activity were then tested in the 3D co-culture model with or without MM-CSCs.
Methods: The viability was assessed by using XTT and MTT assays for RPMI 8226 cells and MM-CSCs, respectively, and by using the CellTiter-Glo 3D assay in the 3D model. The different cell lines within the spheroid were monitored using fluorescent CellTraker dyes. The total cell viability was also monitored by imaging techniques using NucBlue Live for live cells and ethidium homodimer-1 for dead cells within the 3D spheroid.
Results: Withanolide D was one of the most active compound in the 3D co-culture model, supporting results previously reported in the literature regarding the effect on separate cell lines. These findings require further investigation to assess the effect of this compound in the MSC population since they are very important for the tumor dynamic *in vivo*. MSCs change their phenotype when in contact with MM cells by helping the tumor cells to survive and even to become resistant to therapy. Therefore, it is very important to modulate MSCs instead of killing them. Withanolide D was also reported as a potential differentiation agent in MM-CSC. Therefore, it may act as a modulator in MSCs as well.
Conclusions: If confirmed, withanolide D would have a double effect by increasing the probability of success in refractory tumors.

Keywords: multiple myeloma, cancer stem cells, 3D co-culture model, withanolide D, mesenchymal stem cells

P-3

Towards a Better Understanding of Solid Dispersions in Aqueous Environment by a Fluorescence Quenching Approach

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Introduction: Solid dispersions (SDs) represent an important formulation technique to achieve supersaturation in gastro-intestinal fluids and to enhance absorption of poorly water-soluble drugs. Previous researchers have employed fluorescence spectroscopy to study phase behaviour of a supersatured solutions [1]. This study introduces a fluorescence quenching approach together with size-exclusion chromatography to study drug and polymer interactions that emerge from SDs release testing in aqueous colloidal phase. Celecoxib was used as a model drug as it is poorly water-soluble and also exhibits native fluorescence so that quenching experiments were enabled. Different pharmaceutical polymers were evaluated by the (modified) Stern-Volmer model, which was complemented by further bulk analytics. Drug accessibility by the quencher and its affinity to celecoxib were studied in physical mixtures as well as within SDs.
Aims: This work introduces a fluorescence quenching approach together with size-exclusion chromatography to study drug and polymer interactions that emerge from SDs release testing in aqueous colloidal phase.
Methods: Quenching of fluorescence is described by the Stern-Volmer equation and quenching data were presented as plots of F_0/F versus quencher concentration [KI], were F_0 and F are the fluorescence intensity in absence or in presence of the quencher.
Results: The combined analysis of the (modified) Stern-Volmer plots and size-exclusion chromatography enabled unique insight into how the selection of polymer affected the accessibility of drug by the quencher as well collisional affinity in the aqueous colloidal phase. The obtained differences enabled important molecular insights into the different formulations. Knowledge of relevant drug-polymer interactions and the amount of drug embedded into polymer aggregates in the aqueous phase is of high relevance for understanding of SD performance.
Conclusions: The introduction of the fluorescence quenching method was highly useful to study drug-polymer interactions in an aqueous phase. Depending on the polymer, a fraction of drug can obviously be buried in the macromolecule. This reduces free drug in solution, which may lead to lower absorptive flux but also reduces the risk of undesired drug precipitation. The novel fluorescence quenching approach is highly promising for future research and it can provide guidance in early formulation development of native fluorescent compounds.

Keywords: poorly soluble drug, solid dispersion, amorphous formulation, fluorescence quenching, drug-polymer interaction

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Bryophyllum pinnatum Compounds Inhibit the Oxytocin-Induced Rise of Intracellular Calcium Concentration in Human Myometrial Cells

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Introduction: *Bryophyllum pinnatum* is a succulent perennial plant traditionally used in the treatment of premature labour, first in anthroposophic hospitals and, recently, in conventional settings as an add-on medication [1]. Experimental evidence obtained with uterus strips supports this use and suggests that bufadienolides might be responsible for the tocolytic effect [2]. Previous work with myometrial cells showed that *B. pinnatum* leaf press juice (BPJ) inhibits the increase of intracellular free calcium concentration ($[Ca^{2+}]_i$) induced by oxytocin (OT), a hormone known to trigger myometrium contractions [3]. However, it is not known which compounds in BPJ contribute to this inhibition.
Aims: To compare the effects on OT-induced increase of $[Ca^{2+}]_i$ of BPJ, a bufadienolide-enriched fraction (BEF), a flavonoid-enriched fraction (FEF), the corresponding flavonoid aglycon mixture (A-Mix), bersaldegenin-1,3,5-orthoacetate (BO), and the combination of BEF and FEF were tested.
Methods: Human myometrial hTERT-C3 and PHM1-41 cells that had been loaded with a calcium specific fluorescent probe (Fura-2-AM) were pre-incubated with test substances or vehicle controls. Then, cells were stimulated with OT to induce a rapid and transient increase in $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was measured by real-time fluorescence spectrophotometry.
Results: BPJ led to concentration-dependent decrease of the OT-induced increase of $[Ca^{2+}]_i$ in both cell lines ($p<0.0001$), achieving ca. 75% inhibition at a 20 µg/mL concentration. The OT-receptor antagonist atosiban was used as a positive control and also promoted a concentration-dependent effect on $[Ca^{2+}]_i$ in both cell lines ($p<0.0001$). BEF, FEF, BO, A-Mix, and the combination of BEF and FEF led to concentration-dependent decrease of the OT-induced increase of $[Ca^{2+}]_i$ in hTERT-C3 cells ($p<0.05$). BEF (2.2 µg/mL), FEF (17.4 µg/mL), BO (0.04 µg/mL), and A-Mix (0.7 µg/mL), at concentrations corresponding to 20 µg/mL BPJ led to about 25% decrease of the OT-induced increase of $[Ca^{2+}]_i$. The combination of BEF plus FEF led to a decrease of 55.3%.
Conclusions: The data confirm previous observations showing that BPJ promotes a specific and concentration-dependent effect on the OXT signalling pathway. Compounds present in BEF and in FEF seem to have a synergistic effect on the inhibition of the OT-induced increase of $[Ca^{2+}]_i$, which is comparable to the effect of BPJ.

Keywords: *B. pinnatum*, bufadienolides, flavonoids, calcium, *in vitro*

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Phospholipid-based *in situ* Forming Depot Injectable for Sustained Release of a Local Anesthetic

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Introduction: Negatively charged liposome formulations for subcutaneous injection provide a new and innovative platform for sustained drug delivery. Depot formulations offer several advantages such as a reduced dosing frequency or a predictable release profile compared to conventional parenteral formulations.
Aims: Aim of this study is to generate a depot system inducing a controlled aggregation through the interaction of divalent cations with liposomes formulated with negatively charged phospholipids (NCPs). Such a depot could be generated *in situ* and ultimately injected by means of a dual-chamber system. Various NCPs were screened as function of their head groups and acyl chains for their suitability as scaffold for depot formulations. The local anesthetic bupivacaine was used as first drug candidate to be loaded into the liposomes and its release investigated in a physiologically-relevant setup.
Methods: A series of NCPs (25 mol% of total lipid concentration) were formulated with cholesterol (30 mol%) and either the zwitterionic phospholipid L-α-phosphatidylcholine (EPC) or 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (45 mol%). Liposomes were prepared by film hydration method and subjected to freeze-thaw-cycles followed by extrusion. The extent of aggregation in presence of calcium or magnesium was assessed by turbidity (OD_{400}) and by cryo-electron microscopy (cryo-TEM). Bupivacaine (BUP) was encapsulated into liposomes showing the best aggregation profile via ammonium sulfate gradient under different conditions (initial molar drug-to-lipid ratios from 1 to 3). BUP-loaded liposomal formulations were physico-chemically characterized by dynamic light scattering, fluorescence, differential scanning calorimetry (DSC) and cryo-TEM.
Results: Turbidity studies suggested that the aggregation profile of the formulations is dependent on the nature of the phospholipid head group, linked acyl chain and used cation. Particularly, formulations with 1,2-distearoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DSPG), 1,2-dipalmitoyl-sn-glycero-3-phosphate (DPPA) and 1,2-dioleoyl-sn-glycero-3-phosphate (DOPA) showed a noteworthy aggregation tendency [1]. Bupivacaine could be successful encapsulated into the liposomes with final drug concentrations up to 4.2 mg/mL or final drug-to-lipid ratios up to 0.57. As proven by cryo_TEM and fluorescent fusion assays, 150-nm sized BUP-liposomes formed aggregates without fusion. Shifts in transition temperature and appearance of domains in DSC thermograms suggested differences in the binding of the cations to the various NCPs.
Conclusion: Different negatively charged phospholipids showed a desired aggregation profile under the tested conditions and seem to be a candidate for a liposomal depot formulation. To proof this concept, the *in vitro* release profile of bupivacaine from the aggregated liposomes is currently being investigated.

Keywords: liposomes, negatively charged phospholipids, injectable depot formulation

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Saponins from Saffron Corms Inhibit the Secretion of Pro-inflammatory Cytokines at Both Protein and Gene Levels

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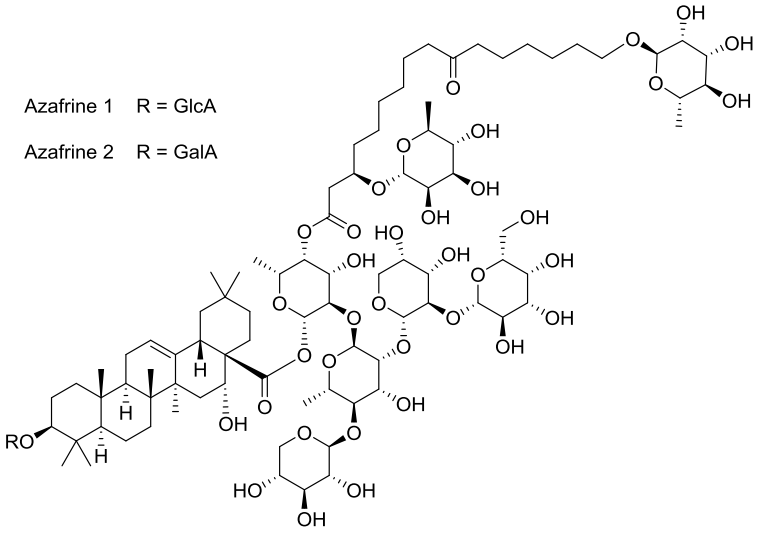
Introduction: *Crocus sativus L.* (Iridaceae) is harvested since ancient times for its characteristic red stigmata, which are widely used as cooking spice. From saffron spice cultivation, large amounts of corms are obtained as by-product.
Aims: This project is placed in the context of the valorization of saffron corms. Preliminary experiments showed anti-inflammatory effects on human keratinocytes. The compounds responsible for the anti-inflammatory effects should be identified and biologically characterized.
Methods: Extracts and compounds were tested on activated human keratinocytes (HaCaT). TNF-α/IFN-γ-induced gene expression and secretion of pro-inflammatory cytokines were determined by a fluorescent-based ELISA. For preparative isolation, a 70% EtOH extract was partitioned between EtOAc, *n*-butanol, and water. The *n*-butanol-soluble fraction was separated by centrifugal partition chromatography (CPC), followed by preparative HPLC on RP-18 and HILIC columns.
Results: A 70% EtOH extract of the corms, and in particular a Diaion HP-20 methanolic fraction inhibited the TNF-α/IFN-γ-induced secretion and gene expression of the chemokines IL-8, MCP-1 and RANTES in human HaCaT cells. The effects were in part stronger than those of the positive control hydrocortisone. A combination of various chromatographic techniques afforded a series of bidesmosidic glycosides of echinocystic acid bearing a fatty acid residue attached to the glycosidic moiety at C-28. The main components were identified as azafrines 1 and 2 (Fig.1) [1]. Saffron saponins significantly inhibited TNF-α/IFN-γ-induced secretion of RANTES in human HaCaT cells at 1 µM ($p < 0.001$). Some of them further lowered TNF-α/IFN-γ-induced gene expression.

Fig.1 Structure of azafrine 1 and 2



Conclusions: Saffron corm extracts and their saponin constituents may have a potential for the development of new cosmetic and/or medicinal products against inflammatory skin conditions.

Keywords: *Crocus sativus L.*, saponins, anti-inflammatory, HaCat, HILIC

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Use of Medication and Recreational Drugs During Pregnancy: A Cross-Sectional Survey in the Canton of Zurich

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Introduction: Use of medication and consumption of recreational drugs during pregnancy have lasting effects on the development of the embryo/foetus. To improve health of their offspring, pregnant women should be aware of those effects and receive adequate support. In addition, health professionals should be well informed about the needs of pregnant women, the evidence level of the most adequate medication, and the consequences of recreational drug use. However, little is known about the overall health condition of pregnant women in Switzerland, particularly with regards medication and recreational drug use.
Aims: The main aim of this analysis was to characterise current medication use among pregnant women and to get an overview of the prevalence of different diseases and symptoms during pregnancy in the Canton of Zurich, which is often assumed to be representative of the population of Switzerland. Moreover, we wanted to investigate the current situation concerning use of recreational drugs among pregnant women in the same setting.
Methods: A questionnaire for pregnant women was created and distributed to participating obstetric clinics and birthing centres in the Canton of Zurich. Data collection took place between August 2018 and March 2019. Thereafter, data sets were manually digitised and a descriptive statistical analysis was performed using IBM® SPSS® Statistics.
Results: In the analysis, 398 questionnaires were included (24.1% of distributed). The most–common chronic diseases among the participating women were allergies (7.8% of total), thyroid disease (5.6%), and headache/migraine (5.4%), whereas heartburn disease, iron deficiency/anaemia and morning sickness were the most frequently mentioned acute diseases/symptoms (corresponding to 18.6%, 16.7% and 14.3% of the pregnant women, respectively). Most women took at least one medication during pregnancy. The medication class that was taken the most were painkillers (33.7%), followed by anti-reflux medicines (20.9%), and antibiotics (11.3%). More than 90% of the participants refrained from taking any recreational drug during pregnancy, 3 out of 4 women who were consuming tobacco before pregnancy quit after conception. Nevertheless, at least 1 in 27 pregnant women consumed tobacco, and 1 in 25 women drank alcohol during pregnancy.
Conclusions: Chronic and acute diseases and discomforts are frequent among pregnant women at least in the Canton of Zurich, and by extension, in Switzerland. While the most often reported diseases/ symptoms were expected, the high prevalence of chronic thyroid disease was surprising. Taken together, our analysis shows that various safe (for mother and child), effective treatments with high levels of patient compliance are urgently needed. In addition, most participants refrained from consuming alcohol, tobacco and illicit drugs revealing a high health-awareness. However, several women mentioned drinking alcohol and/or use of tobacco, suggesting that further preventive work is needed.

Keywords: pregnancy, medication, recreational drugs, behaviour, survey

Medication Discrepancies Between a Standard Medication History and a Best Possible Medication History Performed by a Clinical Pharmacist

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Introduction: Improper documentation of patient's drug therapy at hospital admission often leads to drug related problems during hospitalisation or after discharge. There is little data on the quality of medication histories of newly admitted patients to Swiss hospitals.
Aims: This study aimed to investigate the number and type of medication discrepancies between a standard medication history (SMH) and a best possible medication history (BPMH).
Methods: At the University Hospital Basel, assistant physicians perform the SMHs on admission at the emergency department. During a 2-month period, a clinical pharmacist performed a BPMH on an internal medicine ward in addition to the SMH. The study population consisted of patients, transferred from the emergency department to the internal medicine ward. We performed the BPMH 24 h after the patient's transfer, from Monday to Friday between 12:00 and 14:00 h. This time slot was the limiting factor for the number of BPMHs possible to perform per day. To compile the BPMH we used at least two independent information sources, whereof one was a patient interview if feasible. For each patient we compared the SMH with the BPMH and assessed the number and type of medication discrepancies. We recorded the time to perform a BPMH.
Results: We performed a BPMH in 55 patients (22 females, mean age 66 years) with a total number of 465 drugs. The average number of drugs per patient was 8.5. We identified 227 discrepancies between the BPMH and the SMH, resulting in 4.1 discrepancies per patient on average. Most common types of medication discrepancies were: drugs not documented in the SMH but taken by the patient (N=125, 55%), discrepant dosages (N=64, 28%), documented drugs in the SMH which the patient didn't take (N=30, 13%). We needed on average 35.5 min to perform a BPMH.
Conclusion: This study revealed that SMHs are often incomplete. The performance of a standardized BPMH by a clinical pharmacist seems to be a promising approach to generate an accurate drug list at admission to hospital. The fact that performing a BPMH is very time-consuming might be a limitation for a broad implementation.

Keywords: best possible medication history

P-9

Controlled Opening of Tight Junctions with Short Peptide Inhibitors of Protein Kinase C Zeta

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Introduction: More and more new drug candidates suffer from poor solubility and bioavailability. Drug delivery through the nasal mucosa by opening intercellular tight junctions offers the advantages of avoiding gastrointestinal metabolism and faster onset of drug activity due to the high level of perfusion present in the nasal epithelium.
Aims: Nasal administration of Biopharmaceutics Classification System (BCS) class III drugs and biologics exploiting the paracellular pathway by reversibly and safely modulating epithelial tight junctions (TJs). Several kinases regulate the expression of TJ-associated proteins. One of these is the protein kinase C zeta (PKC ζ). Its inhibition was shown to result in a reversible opening of TJs [1]. Toxicity and kinetic effects are the main data to show the utility of this active excipient.
Methods: We have developed PKC ζ inhibitory myristoylated peptides [2] able to temporarily open TJs of primary human nasal cells grown *in vitro* on semipermeable inserts (Mucilair™). The enhanced diffusion of naloxone and insulin was studied. Potential toxicity of these inhibitory peptides was tested by measuring mitochondrial activity (WST-1), LDH release, hemolysis and ciliary beating frequency (CBF). Reversibility of the effects obtained was also tested, and first *in vivo* studies in wild type and beta-prohibitin knockout mice were performed [3].
Results: Our PKC ζ inhibitors cause around 5-fold higher cell monolayer permeability compared to the control and permit drugs of different molecular weights to diffuse through nasal epithelial cell monolayers *in vitro*. The effect is observed 5 min after application of the peptides. No acute and chronic cell toxicity was observed. Structural modifications of the peptides yielded different effects on permeability modulation. Preliminary *in vivo* results already showed significant reduction in glycemic levels, and are in the process of being confirmed.
Conclusions: Peptidic inhibitors of PKC ζ enhance paracellular permeability of solutes across epithelial cell monolayers *in vitro*. Membrane protein movement can be explored with FRAP studies. First *in vivo* studies gave some good results to continue this proof of concept.

Keywords: tight junctions, permeability, peptide, PKC zeta

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P-10

Particulate Adjuvants for Enhancement of Immunogenicity of Dengue Tetravalent DNA Vaccine

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Introduction: Dengue virus infection is an important public health problem in tropical and subtropical countries. DNA vaccination is considered a potential strategy to overcome this problem. Our partners at Chulalongkorn University in Bangkok have developed 4 plasmid DNA (pDNA) vaccines against each dengue virus strain [1]. Yet, significant immunogenic response is required in order to ensure a sufficient level of protection against this virus, which naked DNA alone cannot provide. In order to overcome this problem and facilitate pDNA delivery, various particulate systems were tested.
Aims: An effective delivery system is needed in order to ensure a safe and effective delivery of the vaccine to the Antigen Presenting Cells (APCs) for both oral and parenteral immunization. Poly-lactic-co-glycolic acid (PLGA) microparticles (1.5-2.5 µm) coated with trimethyl chitosan (TMC) [2], onto which pDNA will be adsorbed are prepared for this goal. The aim is to target DNA vaccine loaded microparticles to antigen presenting cells (APCs), and prevent the particles systemic distribution.
Methods: PLGA (Expansorb® 50-5A, acid-capped and 50-6E, ester-capped) microparticles were formulated using the double emulsion solvent evaporation method. After washing, solutions of TMC at increasing concentrations (50, 75, 100, 200 mg/g) were incubated with the particles for 1 h at RT under moderate stirring. Microparticles are then freeze-dried for 3 days. Microparticles were characterized by laser diffraction and optical microscopy in order to assess size, morphology, and size distribution of the particles. Once coated, their zeta-potential was also assessed both before and after freeze-drying.
Results: No statistically significant difference was observed between Expansorb® 50-6E (ester-capped) and Expansorb® 50-5A (acidic-capped) regarding size and distribution. The D[50] obtained before freeze-drying was 1.67 ± 0.18 µm, no significant difference in size was observed after freeze-drying and coating for all the groups. Zeta-Potential values showed a significant difference between the two coated polymers and between the polymers and the controls ($P<0.0001$). In particular PLGA 50-5A showed a higher charge compared to 50-6E, which will probably produce a better DNA adsorption. A significant difference is also observed for the different TMC concentrations, with a higher zeta-potential value for 100 mg/g and 200 mg/g TMC for the acid-capped polymer.
Conclusion: Overall, the acid capped Expansorb® 50-5A microparticles showed more suitable properties for DNA adsorption than Expansorb® 50-5E. The next step will involve DNA adsorption and release profile, as well as microparticle uptake by antigen presenting cells.

Keywords: DNA vaccine, PLGA, cation microparticles, dengue, trimethyl chitosan

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Development of a Therapeutic Collectin-11 Inhibitor to Minimize Ischemia-Reperfusion Injuries

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Introduction: The complement system is a complex pathway which plays an important role in innate immunity and as a first line of defense against invading organisms, damaged tissues, and maintenance of healthy cell populations. Despite its association with host defense, complement can turn against host cells when dysregulated or excessively triggered. Complement is considered a major contributor to various inflammatory diseases, and its lectin pathway has been implicated in transplantation- and ischemia-triggered clinical conditions. Specific inhibition of this initiation pathway may therefore pave the way for novel therapeutic approaches. Among the potential targets are collectins, a family of C-type lectins which bind pathogen- or damage-associated molecular patterns (PAMPs/DAMPs), thereupon enhancing phagocytosis and/or inducing surface opsonization. Collectin-11 (CL-11, CL-K1) is broadly expressed in multiple tissue types and has been described as an activator of complement via the lectin pathway. It has been previously demonstrated that locally-produced CL-11 plays an important role in both acute kidney injury and late-stage/chronic renal inflammation, and that preventing CL-11 binding to the renal cell surface can reduce neutrophil filtration, C3d deposition, and tubular injury [1-2].
Aims: Given the inherently weak affinities of native carbohydrates for lectins, we have been focused on developing glycomimetic inhibitors of CL-11 with enhanced affinities and improved pharmacokinetic properties.
Methods: We have performed rigid docking and molecular dynamics simulations to gain molecular insights into the mode of CL-11 ligand binding, and have applied this knowledge to construct several glycomimetic libraries. The libraries were then evaluated using biochemical and biophysical methods.
Results: Several glycomimetic libraries have been synthesized and evaluated with the best inhibitors showing improvements of up to 50-fold affinity enhancement.
Conclusions: The development of high affinity CL-11 inhibitors may provide a novel therapeutic for ischemia reperfusion injuries.

Keywords: drug development, complement, glycomimetics, ischemia-reperfusion injury

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Structure-Activity Study and Molecular Insights in the Mode of Action of Complement C3 Inhibitor Cp40

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Introduction: The complement system serves in blood circulation as «first line of defense» against injurious stimuli and invaders. Upon activation, a series of cascading enzymatic reactions leads to an amplification of the response and to pathogen clearance and opsonic cell killing. Yet complement has also gained increasing interest as a potential drug target, since it may be inadvertently triggered on human cells or biomaterial surfaces, thereby contributing to clinical complications in the pathogenesis of various autoimmune, inflammatory and age-related diseases as well as transplant rejection. While the involvement of dysregulated complement activation in inflammatory and autoimmune diseases is now widely recognized [1], so far only one antibody as complement-specific drug has reached the market. The peptidic C3 inhibitor compstatin was originally identified using a phage display approach [2] and several of its derivatives are currently in clinical development. In this presentation we reflect on the development of the picomolar Cp40, a next-generation derivative of the compstatin peptide.
Aim: To identify key interaction determinants of picomolar complement inhibitor Cp40 to its target C3b.
Methods: Solid phase peptide synthesis, structure-activity relationship investigations based on a novel co-crystal structure of Cp40 in complex with C3b; site-specific modifications/ deletions, introduction of nonproteinogenic amino acids and tailor-made building blocks to elucidate the SAR in detail; evaluation of compound affinities by SPR.
Results: Analyzing the co-crystal structure of Cp40 in complex with C3b, we identified key interacting amino acids, namely dTyr-1, (1Me)W-5, Gln-6, Trp-8, Sar-9, Ala-10, His-11, mIle-14 of Cp40. Based on the crystal structure the charged amino acids Asp-7 and Arg-12 don't seem to interact directly with C3b, but mutation of these positions to either charged or hydrophile derivatives revealed a high impact on the off-rate of Cp40.
Conclusion: We were able to identify key interactions of the picomolar complement inhibitor Cp40 to its target C3b by means of structural data from a co-crystal structure as well as tailored SAR studies to quantify the influence of single amino acid positions within Cp40 on on- and off-rate.

Keywords: complement system, peptides, solid-phase-peptide-synthesis, co-crystal structure, structure-activity-relationship

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Nrf2 Pathway Modulation by Resveratrol in Pancreatic Ductal Adenocarcinoma Cells

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Introduction: Pancreatic cancer is nowadays the most lethal gastrointestinal cancer. Due to a lack of symptoms until advanced stage and high aggressiveness of the tumor, the 5-year survival rate is dramatically low and amounts to 9%. Considering that surgical resection is currently the only chance for full recovery and is possible in merely 20% of cases, there is an urgent need for improving chemoprevention strategies and available therapies. Interestingly, it is assumed that a diet rich in vegetables and fruits might be correlated with lower risk of pancreatic cancer development. A natural compound present in broccoli, sulforaphane, has indeed been shown to counteract the aggressiveness of pancreatic cancer through Nrf2 pathway activation [1]. On the other hand, an elevated level of Nrf2 in many types of cancer has been demonstrated to support cancer cell survival, progression and chemoresistance, resulting in poor prognosis [2]. Thus, it is necessary to evaluate the impact of natural compounds present in the diet on pancreatic cancer cells in regards to Nrf2 pathway modulation. Resveratrol, a modulator of the Nrf2 pathway, was chosen for this study.
Aims: To investigate Nrf2 pathway modulation mechanisms used by resveratrol in pancreatic ductal adenocarcinoma (PDAC) cells.
Methods: Two PDAC cell lines, CF PAC-1 and MIA PaCa-2, were treated with resveratrol covering a wide range of concentrations. The cytotoxicity was assessed by the MTT assay and crystal violet staining. Various techniques, such as western blot and immunocytochemistry were applied to study the effect on the Nrf2 pathway by looking at nuclear translocation and downstream protein expression such as HO-1 and NQO1.The antioxidant properties were assessed by Reactive Oxygen Species (ROS) level detection using the DCFHDA fluorescent dye.
Results: The highest concentration of resveratrol (100 µM) significantly decreased cell viability in both cell lines and simultaneously increased expression of Nrf2 pathway downstream proteins. However, the investigation of Nrf2 translocation to the nucleus did not lead to a clear outcome. The antioxidant properties of resveratrol were confirmed in pancreatic cancer cells by the observation of a decreased ROS production in cells pretreated with 10 and 100 µM resveratrol.
Conclusions: Given these results, resveratrol, a compound commonly present in the diet, is involved in oxidative stress response in PDAC cell lines. However, its effect on the Nrf2 pathway is not clear and has to be further investigated.

Keywords: pancreatic cancer, Nrf2 pathway, natural compounds, resveratrol

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Antiproliferative Activity of Compounds Isolated from the Roots of *Ipomoea asarifolia* in Multiple Myeloma

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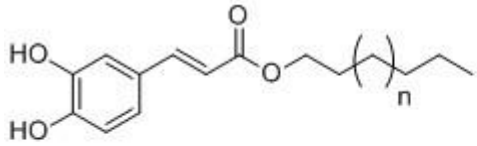
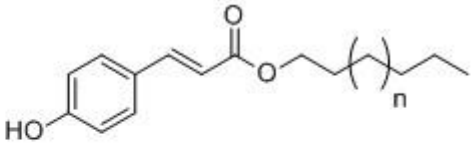
Introduction: *Ipomoea asarifolia* (Desr.) Roem. & Schult. is a plant used in traditional medicine in South America and West Africa for various ailments such as malaria, fever, abdominal pain, snake bites, and wounds [1]. It is also known for its high toxicity in cattle and humans due to the presence of ergot alkaloids in the leaves [2]. During the investigation of plants used in traditional medicine in Niger, 28 plants were screened for their antiproliferative activity against multiple myeloma cancer stem cells (MM-CSCs). These cells are known to be resistant against most current treatments and responsible for relapse. At 20 µg/mL, the dichloromethane (DCM) root extract of *I. asarifolia* inhibited 80% of cell growth. Therefore, this extract was selected for further investigation.
Aim: To isolate and identify compounds responsible for the antiproliferative activity.
Methods: A bioassay-guided fractionation was used to isolate the compounds. First, the crude extract was separated using vacuum liquid chromatography (VLC) in normal phase and fractions were tested at 20 µg/mL against MM-CSCs. Fractions were considered active when cell proliferation was < 50 %. Compounds were isolated and characterized from the active fractions using a reverse phase semi-preparative system HPLC-UV/ELSD, MS, and NMR. They were tested in 4 MM cell lines: MM-CSCs, RPMI 8826, MM.1S, and MM.1R at 50 µM and IC_{50} values were determined.
Results: The VLC of the extract afforded 7 fractions. At 20 µg/mL, fractions 2, 3, and 5 inhibited 60, 90, and 55 % of cell growth, respectively. Based on the HPLC profile, fractions 2 and 3 were selected and yielded compounds **1**-**5** (Fig.1). The IC_{50} values of **1** and **2** (caffeic acid esters) were in the low micromolar range. Compound **1** was more active in MM-CSCs than in RPMI cells with IC_{50} of 0.9 and 10.7 µM, respectively. Compound **2** displayed similar activities in MM-CSCs and RPMI 8226 cells (IC_{50} of 3.8 and 3.1 µM, respectively). Compound **1** did not show any activity at 50 µM in MM.1S and MM.1R, whereas compound **2** had IC_{50} of 11.0 and 13.6 µM, respectively. Compounds **3**-**5** (*p*-coumaric acid esters) were less active with IC_{50} > 25 µM in the four MM cell lines.
Conclusion: Five compounds were isolated from the roots of *I. asarifolia*, 2 of which have not been reported yet (**2** and **5**). Compounds **1** and **2** showed the most promising activities against MM cancer cells. Further investigation are ongoing to isolate compounds from fraction 5.

Fig. 1: Compounds isolated from *Ipomoea asarifolia*



1, n = 10
2, n = 12



3, n = 10
4, n = 12
5, n = 14

Keywords: *Ipomoea asarifolia*, multiple myeloma, bioassay-guided fractionation

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Towards Protecting Biomaterials from Adverse Immune Reactions: Development of a Factor H-Binding Peptide Coating to Prevent Undesired Complement Attack

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Introduction: The complement system is a self-amplifying, fast reacting protein network, largely known for its involvement in host defence pathways. However, its importance in pathologies, such as transplantation and age-related conditions, has been increasingly recognised. Strategies for taming complement attack of host and biomedical surfaces are therefore actively pursued. Some pathogens are exploiting complement regulators, such as the abundant plasma protein Factor H (FH), by recruiting them to their surface and consequently protecting themselves from complement attack. Inspired by this natural approach, our group aims to use synthetic entities to tether FH to cellular or artificial surfaces for therapeutic purposes. Pursuing this idea, a 14 amino acid-long disulphide-bridged cyclic peptide (5C6) was previously discovered by our group through phage display screening. 5C6 showed nanomolar binding affinity to FH and was able to act as a molecular bridge between FH and implant or transplant surfaces when combined with appropriate tethering motifs, leading to a reduced complement activation [1, 2].
Aim: Develop 5C6 towards a preclinical candidate by improving its affinity and stability.
Methods: Compounds were prepared by solid-phase peptide synthesis and further modified using solution-phase reactions. Binding affinities were determined by direct surface plasmon resonance and competitive microscale thermophoresis binding assays.
Results: Four key aspects were identified and addressed. First, truncation of 5C6 at different positions allowed the determination of the minimal binding region. Second, the variation of the size of the macrocycle had a profound impact on activity, enabling us to define the maximally tolerated ring size. Third, the replacement of the disulphide with several other functional groups affected binding affinity to different degrees. Fourth, the replacement of individual amino acids with natural and unnatural amino acids lead to an improved binding affinity of 5C6.
Conclusions: The binding of 5C6 to FH is highly sensitive towards changes in ring size and geometry. Certain amino acids (e.g. H14) are pivotal for binding, whereas other positions (e.g. R5) tolerate substitution or even removal.

Keywords: complement system, factor H, transplantation, biomedical surfaces, peptide synthesis

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A FLIPR Assay for Discovery of $GABA_A$ Receptor Modulators of Natural Origin

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Introduction: Gamma-aminobutyric acid type A ($GABA_A$) receptor modulators are used to treat epilepsy, insomnia, anxiety, and mood disorders. However, currently used drugs lack receptor subtype selectivity and, therefore exhibit different unwanted side effects. Moreover, the scaffold diversity of synthetic drugs and experimental compounds targeting $GABA_A$ receptors is limited. In this regard, recently reported activities of natural products have shown potential for new structurally diverse allosteric modulators. On the other hand, a range of assay formats used for characterization of $GABA_A$ receptor ligands and HPLC-based activity profiling exhibit various drawbacks, which most of these are overcome by Fluorometric Imaging Plate Reader (FLIPR) membrane potential assays.
Aims: A FLIPR assay in 96-well microtiter format utilizing stably transfected Chinese Hamster Ovary cells expressing $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors was established and validated for rapid screening of plant extract libraries and localization of active compounds in complex extracts.
Methods: FLIPR employs a fluorescent-based dye for observation of real-time membrane potential changes associated with ion channel activation. Validation was performed with pure compounds and extracts known to contain allosteric $GABA_A$ receptor modulators.
Results: A protocol for HPLC-based activity profiling was developed, whereby separations of 0.4 to 1.2 mg of extracts on an analytical HPLC column were found to be sufficient for the sensitivity of the bioassay. The protocol successfully localized the activity of known GABAergic natural products, such as magnolol in *Magnolia officinalis*, valerenic acid in *Valeriana officinalis*, and piperine in *Piper nigrum* extracts. EC_{50} values of compounds (magnolol: 4.81 ± 1.0 µM, valerenic acid: 12.56 ± 1.2 µM, and piperine: 5.76 ± 0.7 µM) were found to be comparable or lower than those reported using *Xenopus* oocyte assays.
Conclusions: A FLIPR assay protocol for $GABA_A$ receptor modulation was validated for screening of extract libraries and HPLC-based activity profiling. The FLIPR assay shows significantly higher throughput than current assays with a functional response and has been implemented for an in-house extract library screening. HPLC-based activity profiling of prioritized extracts is currently underway.

Keywords: fluorometric imaging plate reader, $GABA_A$ receptor modulators, assay validation, HPLC-based activity profiling

Searching for Natural Products Targeting Aberrant MAPK/AKT Signaling in Human Melanoma Cell Lines

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Introduction: The incidence of melanoma, the most fatal dermatologic cancer, has dramatically increased over the last decades. This cancer contains the highest mutation rate of all cancers with over 10 mutations/Mb. Studies have shown that more than 50% of malignant melanomas exhibit BRAF V600E mutation, leading to constitutive activation of the ERK pathway. Vemurafenib, a specific BRAF V600E inhibitor, has been approved in 2011 for the treatment of metastatic melanomas [1]. Despite spectacular initial results, drug resistance appears within months. Novel inhibitors targeting aberrant proliferation signaling (ERK and AKT pathways) in melanoma are therefore urgently needed. Nature, especially plants, has always been a prolific source of medicine and natural products continue to provide lead compounds for drug discovery. In cancer chemotherapy, natural products account for over 60% of approved anti-cancer agents [2]. Nature is therefore a promising source for new lead structures.
Aim: Finding new AKT/ERK pathway inhibitors from plant-derived extracts.
Methods: Our natural product lead discovery platform was combined with an innovative high-content screening (HCS) assay. The screen was performed on the well-established human melanoma cell line A2058 (harboring a BRAF V600E mutation and a PTEN deletion), as well as on a patient-derived primary cell line called MM121224 (harboring BRAF V600 and NRAS Q61R mutations). Both lines therefore exhibit high oncogenic ERK and AKT activity. The cell lines were engineered to express genetically encoded biosensors that report on ERK and AKT activity [3], allowing to screen for compounds that would inhibit ERK/AKT activity
Results: A library of 2576 crude extracts was screened. This identified 72 hits on the A2058 line and 257 hits on the MM121224 line. Seventy extracts were chosen for HPLC micro-fractionation and further testing. The so-called activity-profiles that combine the bioassay results with analytical information (UV-ELSD-ESIMS) enabled identification of active compounds in complex extracts. Scale-up and isolation for full characterization is currently in progress.
Conclusions: Our approach allows us to explore the natural product chemical space targeting signaling activities to a more comprehensive fashion than with classic assays. This might enable to identify next generation compounds for targeted therapy of melanoma.

Keywords: melanoma, high-content screening, natural products, HPLC, targeted therapy

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Influence of Water Content on Dielectric Properties and Consequences for Capacitance Based Mass Sensing

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Introduction: Mass-by-capacitance sensors are established Process Analytical Technologies (PAT) tools for capsule filling [1]. These sensors measure the change of an electric field due to a mass that is introduced into the field. There is a linear relationship between the electrical signal and the mass of the pharmaceutical solid. As water has a higher dielectric constant than pharmaceutical solids [2], variations in the water content are expected to influence this measurement. This is especially critical when hygroscopic excipients like microcrystalline cellulose (MCC) are used. Capacitance and apparent resistance are parameters that are typically used as indirect method for describing the dielectric behavior [3]. They are known to behave differently at different frequencies, thus a range of frequencies is tested to see if the influence is different as the frequency increases.
Aim: The aim of this study is to quantify the impact of water content on capacitance and apparent resistance at different frequencies using a LCR meter.
Methods: Two hygroscopic excipients, Vivapur® 101 (JRS Pharma GmbH & Co KG) and maize starch (Roquette) and a non-hygroscopic compound, paracetamol, were used for the experiments. The electrical parameters capacitance and apparent resistance were measured using a LCR meter (IM3536, HIOKI, Japan) with an electrode (16451B, Agilent, US) using a range of frequencies from 100 to 8000 kHz. The experiments were performed at 3 different levels of relative humidity (11, 45, and 66%), 3 replicates per every relative humidity level. The pharmaceutical solids were equilibrated in a flow-through cell at the respective humidity for 5 h before the measurement.
Results: Both, capacitance and apparent resistance, have similar qualitative behaviours with changes in the moisture content. They both increase with increasing moisture, but the relative increase does not vary with the frequency. The capacitance however is approx. twice as sensitive to the water content than the apparent resistance in the range of 0.1-13.2% water content (22.9% increase of capacitance per 1% increase in water content *vs.* 11.6% increase of apparent resistance per 1% increase in water content). This is interesting, as a simultaneous determination of both capacitance and apparent resistance would theoretically allow estimating the water content. Additionally, it is possible to correlate results of the electrical parameters from different compounds with the moisture content of the pharmaceutical solids and the results obtained by Loss on drying (LOD) experiments..
Conclusions: The relative impact of water on the measurement of capacitance and apparent resistance is independent of the frequency in the investigated range of 100 to 8000 kHz. The magnitude of the influence however is different for capacitance and apparent resistance (22.9% increase of capacitance per 1% increase in water content *vs.* 11.6% increase of apparent resistance per 1% increase in water content). Therefore, based on the obtained results, a simultaneous measurement of both parameters would reveal the water content in addition of the mass of the pharmaceutical solids or any changes thereof.

Keywords: capacitance, resistance, moisture content, sensor, PAT

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Core-Shell Drug Delivery System for Single-Dose Pandemic Influenza Vaccines

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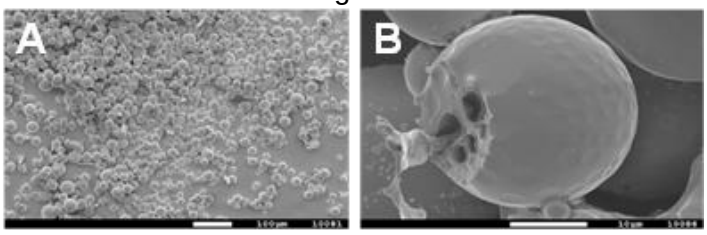
Introduction: The benefits of a single-dose influenza vaccine over a multi-immunization regimen are significant in terms of population coverage, cost and time reduction in a pandemic situation. It is required that a single-dose elicits a comparable immune response to a customary «prime and boost» regimen. To mimic the «boost» in a single-dose vaccine formulation, a delivery system for whole inactivated H5N1 influenza (WH5N1) is needed that maintains antigenicity of the encapsulant and allows for a delayed release profile.
Aims: To develop a delivery system for WH5N1 based on poly (lactic-co-glycolic acid) (PLGA) microparticles containing a hydrogel alginate core. A promising approach, as the core facilitates a desirable encapsulation environment for influenza vaccine antigen and the biodegradable polymer shell allows for controlled-release.
Methods: A two-step method for the manufacture of PLGA-Alg microparticles is possible using double-emulsion-solvent evaporation combined with ionotropic gelation [1, 2]. The vaccine was combined with sodium alginate solution (Alg) (3.5% *w/v*) then emulsified in solvent (DCM) containing PLGA (10% w/v) by homogenization. The primary emulsion was then added to a PVAL (1% *w/v*) solution containing $CaCl_2$ (100 mM) and homogenized shortly. The influx of Ca^{2+} ions cause the crosslinking of the alginate gel. The double emulsion was stirred for 4 h, allowing the solvent to evaporate and subsequent solidification of PLGA. Microparticles are then washed and collected by centrifugation and lyophilized for further analysis.
Results: Particle size measurements by mastersizer showed the procedure was repeatable (n=4), measuring a mean volumetric size D(4;3) of 38.6 µm, with span 1.8. Morphology visualized with SEM revealed homogenous spherical particles with dimple-like indentations on the surface. After lyophilization, the cross-sectional morphology of microparticles could be observed with a double membrane inside the PLGA matrix, signifying the presence of Ca-Alg hydrogel cores in the microparticles. This was further confirmed by fluorescence labeling and confocal imaging.

Figure 1. SEM images of (A) Overview of Alg-PLGA microparticles, (B) the interior of a microparticle where core membrane of cross-linked alginate is visible



Conclusions: A repeatable manufacture procedure for alginate-core PLGA microparticles was completed. The hydrogel core was confirmed by SEM and confocal imaging. Subsequent work will implicate the development of analytical methods to determine the stability and antigenicity of the vaccine after encapsulation. The *in vivo* release profile and the concurrent immune response will be tested *in vivo* using C57/BL6 mice, the humoral response measured by neutralizing antibody titers and cellular response in T-cells by ICS/FACS.

Keywords: pandemic influenza, delayed release, hydrocore microparticles, single-shot

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Acknowledgements:
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Preclinical Investigation of Targeted Radionuclide Therapy of Prostate Cancer: Comparison of ^{161}Tb-PSMA-617 and ^{177}Lu-PSMA-617

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Introduction: ^{177}Lu is a β^-–emitter currently employed in clinics for targeted radionuclide therapy of prostate cancer using the prostate-specific membrane antigen (PSMA)-targeting ligand PSMA-617 [1]. In spite of promising results, patients often experience relapse due to surviving cancer cells.
Aims: The aim of our research was to address this situation by using ^{161}Tb as a potentially more powerful alternative to ^{177}Lu. ^{161}Tb emits not only β^-–particles but also low-energy conversion and Auger electrons, which may effectively kill micrometastases.
Methods: ^{161}Tb-PSMA-617 and ^{177}Lu-PSMA-617 were compared with regard to their *in vitro* properties. Cell viability (MTT assays) and survival assays (colony forming assays) were performed using PSMA-positive PC-3 PIP and PSMA-negative PC-3 flu prostate cancer cells. Both radioligands were investigated in biodistribution and imaging studies using tumor-bearing-mice. Preclinical therapy studies in mice were conducted with the radioligands at activities of 2.5, 5.0 and 10 MBq per mouse in PC-3 PIP tumor-bearing mice.
Results: The chemical and pharmacokinetic properties of the radioligands were equal, independent on whether ^{161}Tb or ^{177}Lu was employed. ^{161}Tb-PSMA-617 was, however, significantly more effective than ^{177}Lu-PSMA-617 in reducing prostate cancer cell viability and survival *in vitro* [2]. These findings were in line with theoretical calculations, which predicted a 3-4-fold increased dose deposition to cancer cell monolayers or single cancer cells when using ^{161}Tb as compared to ^{177}Lu. *In vivo*, ^{161}Tb-PSMA-617 inhibited tumor growth in mice and increased their survival significantly more effectively than ^{177}Lu-PSMA-617 without causing early side effects [2].
Conclusions: Our investigations indicate an advantage of using ^{161}Tb-PSMA-617 as compared to ^{177}Lu-PSMA-617 for the treatment of metastatic prostate cancer. To confirm the anticipated superiority of ^{161}Tb over ^{177}Lu, the respective radioligands will be investigated in more detailed preclinical studies using patient-derived xenograft (PDX) mouse models that reflect the clinical situation better. If our hypothesis of the superior effect of ^{161}Tb will be confirmed, the concept is likely to be translated to clinical trials.

Keywords: targeted radionuclide therapy, PSMA-617, ^{161}Tb, ^{177}Lu, prostate cancer

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Evaluation and Optimization of HPTLC and Assay Methods for Aurantii Amari Flos and Aurantii Dulcis Flos

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Introduction: The European Pharmacopeia contains a monograph for bitter-orange flowers [1] but the TLC test on sweet-orange flowers as well as the photometric assay method are not satisfactory in respect of specificity and reproducibility. A literature search revealed naringin, neoeriocitrin and neohesperidin as possible candidates for distinct substances for bitter-orange flowers [2]. On the other hand, no publication was found on the constituents of sweet-orange flowers, therefore hesperidin and narirutin were tested based on the substances known from the peel and flesh of sweet-oranges [3].
Aims: The current work focuses on the optimization of the identification and test method by HPTLC with the goal to clearly distinguish bitter- and sweet-orange flowers and to detect mixtures of them. In a second part, a new assay method by UHPLC for the main flavanone-glycosides, contained in both herbal drugs, was developed.
Methods: The existing HPTLC identification method was slightly improved, with a mobile phase of ethyl acetate:water:formic acid (65:20:15: *v/v/v*) yielding the best results. A combination of naringin and rutin was selected as reference and intensity marker. For the determination of content, an UHPLC gradient method with 0.1% formic acid and acetonitrile on a BEH C18 column (100 mm x 2.1 mm i.d., 1.7-µm particle size) provided satisfactory separation. For both methods, the samples were extracted with methanol 50% *v/v*.
Results: Based on the chromatographic profiles (HPTLC, UHPLC), the samples could be divided into 3 relatively homogeneous groups. All bitter-orange flowers showed a similar fingerprint, whereas the sweet-orange flowers formed 2 groups that could be correlated to their origin. Nevertheless, the differences in the HPTLC pattern between bitter- and sweet-orange flowers seemed too small to detect small amounts of sweet-orange flowers in a batch of bitter-orange flowers. The opposite way should be the smaller problem due to the distinct red zone due to neoeriocitrin, together with 1 additional olive green and 2 yellow zones present in the HPTLC of the bitter-orange flowers (UV 366 nm, 60 min after NP/PEG). In conjunction with the developed UHPLC method, a yellow zone observed under UV 366 nm as well as in daylight was identified as rutin and was only present in the samples of sweet-orange flowers. The results of the assay showed a total flavonoid content of 12.84 ± 2.16 % *w/w* (calculated as naringin, mean ± s) for the bitter-orange flowers, consisting of neohesperidin (5.8 ± 1.18 % *w/w*), naringin (3.86 ± 0.77 % *w/w*) as well as neoeriocitrin (0.69 ± 0.12 % *w/w*). Hesperidin (0.68 ± 0.13 % *w/w*) and narirutin (0.64 ± 0.18 % *w/w*) were also detected. The total flavonoid content in the sweet-orange flowers was found to be 1.18 ± 0.05 % *w/w* with the main constituents being hesperidin (0.58 ± 0.02 % *w/w*), rutin (0.38 ± 0.015 % *w/w*) and small amounts of narirutin (0.04 ± 0.001 % *w/w*). The validation of the assay method showed good linearity and specificity. However, the method precision in form of its repeatability was not yet satisfying and requires additional experiments addressing the sample preparation procedure.
Conclusions: The content of total flavonoids in the bitter-orange flowers was about 13 times higher, compared to sweet-orange flowers. Based on their chromatographic profiles, 2 types of sweet-orange flowers were observed, depending on their origin. Rutin was only detected in sweet-orange flowers and may allow detecting a possible falsification of bitter- with sweet-orange flowers. The developed methods will lead to improved Pharmacopoeial monographs for bitter- and sweet-orange flowers.

Keywords: aurantii dulcis flos, aurantii amari flos, flavonoids, HPTLC, UHPLC

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In Vivo Stability of Biotherapeutics

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Introduction: Significant efforts are made to characterize molecular liabilities and degradation of the drug substance and drug product during various product life-cycle stages. The *in vivo* fate of a biotherapeutic is usually only considered in terms of pharmacokinetics and pharmacodynamics. However, the environment in the human body differs substantially from that of the matrix (formulation) of the drug product and may impact on the stability of an administered biotherapeutic.
Methods, Results: *In vivo* stability: Stabilizing excipients used in protein formulations are expected to undergo more rapid distribution and dissociation *in vivo,* compared to a protein as a highly charged macromolecule. Thus, *in vivo* stability may significantly differ from shelf-life stability. *In vivo* degradation of the biotherapeutic may alter efficacy and/or safety characteristics such as immunogenicity.
Conclusion: Studying the stability of a biotherapeutic in the intended body compartment can de-risk drug development in early stages of development by improving the selection of better clinical lead molecules.

Keywords: *in vivo* protein stability, human body fluids, *in vitro* model, biotherapeutic, developability

Hidden in the Woods: Quality Markers of Conifer-Derived Essential Oils

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Introduction: The needles of *Pinus* distributed all over the world are source of precious essential oils (EOs) and are used in antiseptic treatment of respiratory infections, mostly administered through inhalation. EOs are mixtures of natural chemical substances mainly categorized as monoterpenes, sesquiterpenes, and their oxygenated derivatives. Genuine EOs in this study are obtained from different *Pinus sylvestris* (*P. sylvestris)* worldwide. *P. sylvestris* is an evergreen conifer tree and occurs naturally in the northern hemisphere. The chemical fingerprint of these genuine EOs can be analyzed to characterize authentic EOs and to avoid the possibility of confusion.
Aims: The present work aimed to chemically characterize in details genuine EOs of *P. sylvestris* from different origins by applying gas chromatography with flame ionization detection (GC-FID) for fingerprinting and gas chromatography mass spectrometry (GC-MS) to identify the fingerprints. Additionally, 8 selected compounds of the EOs should be determined by absolute quantification.
Methods: EOs obtained by steam distillation of needles of genuine *P. sylvestris* from different origins were analyzed and characterized with regard to monoterpene and sesquiterpene profiles using GC-FID. A GC-FID method for the absolute quantification (multi-point calibration) of 8 compounds was developed and validated according to international guidelines.
Results: The chemical fingerprint of the genuine EOs of *P. sylvestris* could be classified into monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. Monoterpene hydrocarbons were predominant with α-pinene as main compound. Additionally, the genuine EOs showed a large sesquiterpene fingerprint containing γ-cadinene and germacrene-d-4-ol, which are used as quality markers. The method for the absolute quantification was successfully validated and α-pinene, camphene, β-pinene, 3-carene, limonene, bornyl acetate, β-caryophyllene, and borneol were determined.
Conclusion: For a proper evaluation of the quality of genuine EOs of *P. sylvestris* it is recommended to acquire chromatographic fingerprints and perform absolute quantification.

Keywords: *Pinus sylvestris*, essential oil, gc and gc-ms fingerprints, quantification, quality control

Printfills: 3D Printed Systems Using Fused Deposition Modelling and Injection Volume Filling

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Introduction: Fused deposition modelling (FDM) has become a very attractive technology for development of pharmaceutical systems. Drug release profiles from FDM printed tablets are easily controlled by varying factors such as geometry, polymer selection or drug load. However, FDM has the limitation of the impossibility to print thermally sensitive drugs and the need to incorporate the drug to the polymeric filament as a previous step of manufacture. We hypothesize that the integration of FDM with Injection Volume Filling (IVF), which allows incorporating solutions/dispersions at room temperature to the extruded scaffold, could offer an easy, automatized and versatile technology to manufacture tailored drug delivery platforms [1].
Aims: The aim of this work was to design and characterize colon-specific drug delivery systems manufactured in a simple and automated 3D printer which combines two different 3D printing technologies FDM and IVF. This new kind of printed pharmaceutical dosage forms have been called *printfills*: printed systems filled with a liquid or semisolid.
Methods: Polylactic acid was used as printer filament for FDM (Leon3D, Spain). Anhydrous theophylline (Acofarma, Spain) and Eudragit FS30D (Evonik, Germany) were used as model drug and delaying release polymer, respectively, for IVF. Printfills were manufactured with a REGEMAT 3D V1 printer (Spain) with FDM and IVF. A hydroalcoholic drug gel was injected in the extruded structure and Eudragit FS30D dispersion was incorporated into the top layer of the structure. Printfills were characterized from a physical and biopharmaceutical point of view, including SEM microscopy (FEI Teneo) and drug release modeling.
Results: 3D printed systems have been successfully performed using FDM and IVF (Fig.1). Results from drug release studies performed at different pH confirm the ability of printfills for colon-specific drug delivery. SEM microphotographs of printfills show the sealing of the structure in the perimeter and the homogeneity of the colonic film formed in the upper side. The obtained results indicate that, after the lag time, drug is released with an intermediate kinetics between zero order and diffusional kinetics, as shown by Korsmeyer time exponent (n=0.8749).

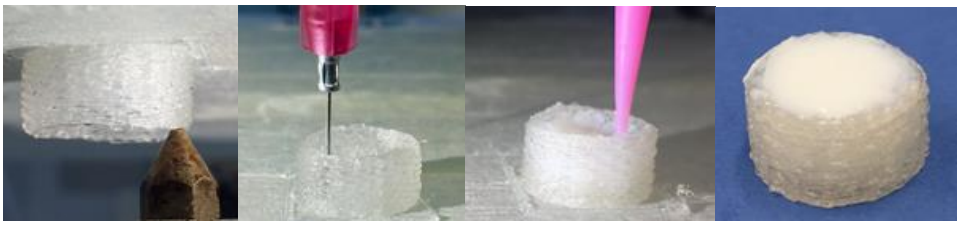

Fig. 1 From left to right: FDM extruder (PLA), IVF injecting drug–loaded gel, IVF injecting the delaying release polymer and final printfill obtained [1]

Conclusions: Pharmaceutical dosage forms have been manufactured for the first time with a 3D printer combining FDM and IVF. The integration of these two techniques allows an easier incorporation of drug/excipient liquid systems to the extruded scaffold at room temperature, avoiding other intermediate processes. *In vitro* studies show the ability for colon specific drug delivery of the performed printfills [1].

Keywords: 3D printing, fused deposition modelling, injection volume filling, colon specific release

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Development and Evaluation of 3D Printing Filaments for Oral Drug Delivery Systems

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Introduction: 3D printing has proved to be one of the fastest growing technologies due to its huge variety of applications, as a platform to develop new pharmaceutical systems. One of the most used techniques for 3D printing is the FDM (Fused Deposition Modelling) for its multiple advantages such as low cost, simplicity and versatility. In order to obtain pharmaceutical systems, these filaments can be pre-loaded extruding a blend of drug and polymer.
Aims: The objective of this work was the development and characterization of polymeric filaments loaded with drug for its use in FDM 3D printing technology. For this reason, filaments made of a binary mixture of polyurethane and ibuprofen were analysed from a physical and biopharmaceutical point of view.
Methods: Pellets of polyurethane Tecoflex^{TM} EG72D (Lubrizol, Spain) were frozen using liquid nitrogen to mill them (Retsch, Germany). Binary mixtures with 20-50% *w/w* of ibuprofen (Acofarma, Spain) were made and filaments were obtained by a single screw extruder (Filastruder, United States). Filaments and powder mixtures were analyzed by differential scanning calorimetry (DSC) (Setaram-DSC 131, France). Filaments were printed on a 3D printer (Regemat 3D S.L., Spain) and drug release studies were carried out in a USP II dissolution apparatus (Sotax, Switzerland).
Results: Selecting adequate extrusion temperatures all mixtures were extruded successfully achieving filaments with suitable diameter to be printed. Filaments showed different physical characteristics depending on the mixture employed. DSC studies revealed the physical changes suffered by the drug after extrusion, going from crystalline to amorphous state in the solid dispersion inside the filaments. Drug release from the filaments exhibited a diffusional kinetics, showing higher drug release rates as the drug concentration increases. The drug percolation threshold has been estimated between 31.5% and 35.0% *v/v* of drug according to the percolation theory. 3D printed systems were obtained with the filaments with suitable characteristics showing an increase of 20% of drug release respect to their respective filaments. This effect can be attributed to the higher surface area exposed to the medium, due to the smaller diameter of the strand forming the printed system.
Conclusions: 3D printed filaments can be successfully obtained with mixtures of polyurethane and ibuprofen, controlling the extrusion temperature as the main critical factor of the process. Filaments were solid dispersions where a physical state change of the drug has been confirmed by DSC. Drug release from filaments shows a critical point around 33 % *v/v* of drug. The performed 3D printed systems provided a higher surface area exposed to the release medium than the filaments, leading to a faster drug release.

Keywords: 3D printing, filament, extrusion, drug release, percolation threshold

Acute Reinforcement, But Little Adaptive Behavior With Ketamine

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Introduction: Ketamine, a bona fide NMDA antagonist, is approved as antidepressant with a fast onset. Ketamine is also recreationally abused for its dissociative effects. Concerns over ketamine abuse could impede clinical use, yet its addiction liability has not been directly investigated.
Aims: To assess the abuse potential of ketamine in a mouse model, in comparison to the well-studied psychostimulant cocaine.
Methods: We used a novel genetically encoded sensor for dopamine (DA) to measure DA levels in the nucleus accumbens (NAc) of mice in response to ketamine injections. We also assessed behavioral reinforcement and drug-adaptive plasticity *ex vivo*.
Results: A single intraperitoneal injection of ketamine (30 mg/kg) induced DA transients comparable in magnitude to cocaine (15 mg/kg) but of shorter duration. Ketamine reinforced lever pressing for intravenous infusion and led to conditioned place preference, although the effect size was smaller than with cocaine. Acute hyperlocomotion was comparable between ketamine- and cocaine-treated mice, however, unlike cocaine, ketamine did not induce behavioral sensitization. In parallel, drug-evoked synaptic plasticity of excitatory afferents onto D1-MSNs, a hallmark of early neural adaptation to all addictive drugs, was not observed with ketamine.
Conclusions: We conclude that ketamine at doses typically used in recreational setting is reinforcing but does not potentiate accumbal afferents, likely by inhibiting NMDA receptors, thus limiting drug-adaptive behavior.

Keywords: addiction, dopamine, ketamine, self-administration, synaptic plasticity

High Throughput Screening of Inhibitors of the Cytoplasmic DNA Clustering Function of Human Barrier to Autointegration Factor

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Introduction: The efficient delivery of DNA is a prerequisite for the translation of gene therapy into the clinic. Non-viral strategies to transport DNA into the nucleus of mammalian cells are preferred over viral ones given their lower safety risks. However, they are usually associated with much lower transfection efficiencies. This is due in part to the presence of several barriers, which sequester most of the DNA before it can reach the transcription machinery in the nucleus. One of these barriers, that received little attention but might greatly impact the nuclear uptake, is a cytoplasmic defense system against exogenous DNA which recognizes and clusters DNA in the moment it enters the cytoplasm. The main proteins involved in cytoplasmic clustering of DNA is Barrier to Autointegration Factor (BAF) and its direct binding partners the LEM (LAP2β, emerin, MAN1) domain containing transmembrane proteins.
Aim: Our aim is to find small molecule inhibitors against the LEM-BAF-DNA interaction chain to increase cytoplasmic DNA solubility and, eventually, its availability for nuclear uptake.
Methods: We have set-up an ELISA-type assay in which the LEM domain of human emerin is immobilized on the bottom of a multi-well plate. Then wild type BAF is added together with fluorescently labelled DNA. Active BAF binds to both the LEM-domain and DNA resulting in DNA retention. In the presence of an inhibiting compound, the interaction chain LEM-BAF-DNA is broken and the fluorescence signal decreases. With this assay, we performed a High-throughput Screening (HTS) of the Prestwick Chemical Library, the Protein-Protein Interaction Library, the Natural Products Library, the Chemical Diversity Collection and the Maybridge HitFinder Collection (30,000 compounds in total). Subsequently, we re-validated the primary hits with further *in vitro* and cell culture assays.
Results: The HTS resulted in 4 compounds that showed significant inhibition of DNA retention. Three compounds – among them rabeprazole – contained sulfide groups and our data indicate that their activity is based on the formation of disulfide bonds with the BAF cysteins. Accordingly, inhibition could only be observed under oxidizing assay conditions. Under reducing conditions, the inhibitors were not active and no significant effect on transfection efficiency could be observed in cell culture experiments.
Conclusion: A robust HTS assay to test for the interactions between LEM domain, BAF and DNA was developed. Several inhibiting compounds were identified. However, further screening is required to find inhibitors that are active under the reducing conditions of the cytoplasm.

Keywords: barrier to autointegration factor, DNA transfection, nuclear delivery, high-throughput screening, small molecule inhibitors

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Safe-by-Design Concept Applied to Polymeric Biomaterials for Drug Delivery: Chitosan-Insulin Nanoparticles as a Case Study

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Introduction: Polymeric nanobiomaterials have been introduced for diagnostics and drug delivery. However, the number of marketed products based on nanobiomaterials remains limited due to the regulatory assessment of efficacy and safety of such materials yet to be defined. Pre-clinical data on polymer chemistry, sourcing, nanoparticle (NP) preparation methods at lab scale, physicochemical characterization, *in vitro* characterization, cytotoxicity, immunotoxicity, animal and human toxicology, and environmental risks are to some extent available in the literature. However, these data are not well structured, characterization assays not harmonized or validated, and scale-up with in-process controls not established. Correlations between physicochemical properties of NPs and clinical outcome of their use (critical quality attributes, CQA) are not established. The GoNanoBioMat project aims to support SMEs (small and medium size enterprises), suppliers, and academia with the development of polymeric nanobiomaterials for drug delivery. Chitosan is one of the most studied biopolymers, however, there is no standardization of its characteristics and biological activity. In the framework of the GoNanoBioMat project this study assembled information on protocols used to prepare chitosan NPs encapsulating insulin (Cs-Ins NPs) as a model protein drug, and on information on the immunotoxicological response to Cs-Ins NPs.
Aims: The aim was to demonstrate the lack of chitosan NPs preparation systematization, to compare and analyze chitosan-insulin nanoparticle preparation protocols, and to discuss the importance of studies on potential immune responses elicited against Cs-TPP NPs.
Methods: PubMed and Science Direct were consulted using MESH *chitosan, immune activity, gelation, insulin, encapsulation,* and *adjuvant*. Focusing on ionotropic gelation, using tripolyphosphate (TPP) as crosslinker, insulin was chosen as a model for protein encapsulation into those nanoparticles.
Results: Several important differences between the available data and protocols for Cs-Ins NPs preparation exist. Among them the most basic information such as chitosan molecular weight, degree of deacetylation, viscosity, and purity. Other differences in the preparation of Cs-Ins NPs were the excipient source, concentration of chitosan, TPP, and insulin used, and the sequence of preparation steps. As a result of these differences, Cs-Ins NPs have different properties. It is therefore not possible to directly compare NPs obtained by these studies. In addition, no information was available on potential immune responses elicited against Cs-Ins NPs.
Conclusions: The aim of GoNanoBioMat is establishing a safe-by-design guideline that allows selection of excipients and protocols most appropriate for the intended purpose. Future studies comprising chitosan NPs should include better characterization of chitosan to understand how its properties influence NPs characteristics and insulin encapsulation. The next step will be establishing a correlation between chitosan NPs properties and their immunostimulant activity.

Keywords: chitosan, immune activity, gelation, insulin, encapsulation

Development of a Sustained-Release Depot Formulation of Buprenorphine for Pain Relief in Experimental Animals

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Introduction: Buprenorphine is a fast-acting semisynthetic opioid derivative, frequently used in veterinary medicine for post-surgical pain relief in mice and rats. Due to its short half-life, repeated injections are required to maintain analgesic effect. Consequently, animals are exposed to increased levels of stress and might suffer additional pain.
Aims: As no depot formulation is available on the European market, the aim of this project is to develop a sustained-release formulation of buprenorphine to prolong the analgesic effect after surgical intervention in rodents.
Methods: The developed sustained-release formulation is based on drug-loaded microparticles. Various formulations were studied regarding their influence on *in vitro* drug release, prior to *in vivo* testing. The most promising formulation was administered subcutaneously to 32 female C57BL/6J mice to investigate the pharmacokinetic profile of buprenorphine in serum over a period of 72 h. Analgesic action was further assessed up to 48 h after a single injection using a thermal sensitivity test (hotplate assay) and compared to the standard formulation Temgesic®.
Results: Serum concentrations indicate that the developed depot formulation provides an adequate analgesic effect for at least 24 h. After 48 h, serum buprenorphine concentrations drop below the threshold where an antinociceptive effect can be expected. The hotplate assay confirmed a significant increase in withdrawal latency for the sustained-release formulation after 2, 12 and 24 h when compared to the baseline. Furthermore, a significant increase in withdrawal latency for Temgesic® could only be shown for the first time point of 2 h. Afterwards, no significant difference to baseline was detected, confirming the short duration of action of less than 12 h.
Conclusions: A promising sustained-release depot formulation of buprenorphine based on biodegradable microparticles was developed. Serum concentrations and thermal sensitivity assays show an analgesic efficacy of at least 24 h, significantly prolonging the effect compared to the marketed standard formulation.

Keywords: microparticles, sustained-release, buprenorphine

Development of a Leech Protein that Inhibits the Classical and Lectin Pathways of the Complement System

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Introduction: The complement system is comprised of more than 50 proteins serving as a first line of defence mechanism against pathogens. While pivotal for homeostasis, inappropriate complement activation is a detrimental contributor to various clinical conditions. A better understanding of the initiation of the cascade and its involvement in diseases may therefore pave the way for novel therapeutic approaches [1]. Complement can be activated via various pathways; the classical (CP) and lectin pathway (LP) are both mediated by serine proteases, i.e. C1s/C1r (CP) and MASP1/MASP2 (LP). These pathways have a vital role in recognizing pathogens but also contribute to autoimmune diseases such as haemolytic anaemia and ischemia-reperfusion injuries. *Haementera ghiliani*, the Giant Amazon leech, produces a protein called BD001 inhibiting both C1s and MASP1/2 as part of its immune evasion strategy [1,2]. The protein of interest could therefore serve as a lead structure for future therapeutics.
Aim: Express correctly folded and active BD001 in bacteria and determine inhibitory potential.
Methods: The BD001 gene was amplified using PCR, ligated into the pET15b vector and transformed into the bacterial strain *Rosetta-gami*. The protein was purified via metal ion affinity chromatography via its histidine-tag. Expression yield and purity were tested on SDS-page gel. Inhibitory potential was evaluated in different biochemical assays. In a C4 cleavage assay, C1s and its natural substrate C4 were incubated and cleaved C4a visualized by SDS-PAGE. In a chromogenic substrate assay, the ability of different inhibitors to prevent C1s-mediated cleavage of a chromogenic substrate was assessed by spectro-photometry. Finally, in a haemolytic assay, IgM-coated sheep erythrocytes were incubated with 1% normal human serum and a concentration series of inhibitors. Erythrocytes lysis was determined by measuring the absorbance of free haemoglobin.
Results: We were able to express this highly challenging 122 amino acid long protein, featuring 10 disulphide bridges, in the bacterial strain *Rosetta-gami*. In the bacterial system high yields and fast production as well as high purity could be achieved. After successful purification the protein showed potent activity in all biochemical assays.
Conclusions: Not only we were able to produce the protein in a prokaryotic system, but also determine its activity and therefore its inhibitory potential. Our findings establish BD001 as an interesting lead structure for drugs treating diseases such as autoimmune haemolytic anaemia or ischemia-reperfusion injuries (e.g., transplantation or stroke).

Keywords: complement system, immunology, protein therapeutics

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Lin28 Inhibition by a Small Molecule Led to Insulin Resistance and Increased Ketogenesis

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Introduction: The RNA-binding protein Lin28 and its paralogue Lin28b play critical roles in embryonic development, tumorigenesis, pluripotency, and energy utilization. Lin28 proteins bind to the terminal loops of most let-7 precursors and block their processing into mature miRNAs. Thus, Lin28 proteins de-repress the expression of let-7 target genes. Lin28 proteins also have let-7 independent roles. They bind directly to multiple mRNAs, amplifying their translation. Aberrant expression of Lin28 and let-7 has been observed in many human malignancies. Increasing evidence suggests that the Lin28/let-7 axis is physiologically required for normal glucose homeostasis [1]. Lin28 and Lin28b can increase the expression and sensitivity of components of the insulin-PI3K-mTOR signalling pathway and numerous metabolic genes are direct let-7 targets [1].
Aims: In the current project, we sought to examine the effect of the small molecule Lin28 inhibitor 1632 [2] in a mouse model of prostate cancer and to characterize the pharmacological properties of this compound.
Methods: Xenograft mice of human prostate cancer were intraperitoneally injected with 1632 and *in vivo* measurements (tumour growth/weight, glycaemia) were performed. Myoblast C2C12 and hepatoblastoma HepG2 cells were used as relevant *in vitro* systems. Transfections with plasmids or siRNAs were done with Lipofectamine 2000. Immunoblotting and qPCR were used to study the protein and mRNA levels of genes of interest. A ketone body assay was used to measure the concentration of 3-OH butyrate in mouse serum, and cell growth medium. A lactate assay was used to measure lactate concentration in cell growth medium.
Results: 1632-treated mice did not display any statistically significant change in their tumour growth and weight, but they showed elevated blood glucose levels. In light of the role of Lin28 in glucose metabolism, we analysed the protein levels of Lin28b, as well as some central components of the insulin-PI3K-mTOR pathway (IRb, P-Akt/Total Akt) and we found that they are down-regulated in skeletal muscle from 1632-treated mice. In the C2C12 cell culture system, gain and loss of function of Lin28 confirmed a role in fine-tuning the PI3K-mTOR pathway. Subsequent treatment of mice with 1632 showed a 3-fold increase in the serum concentration of 3-OH butyrate in 1632-treated mice over their mock-treated littermates. To investigate the potential role of Lin28 in ketogenesis, we have treated HepG2 cells with siLin28b and 1632. 1632-treated HepG2 cells displayed increased 3-OH butyrate secretion in their growth medium, along with decreased Lin28b and Insulin Receptor β levels.
Conclusions: Lin28b inhibition by a small molecule led to increased glycaemia, accompanied by repression of the insulin-PI3K-mTOR pathway in xenograft mice of prostate cancer. Non-tumour bearing mice treated with the same compound exhibited increased ketogenesis. This project offers the opportunity to gain a better understanding of how Lin28 participates in crucial metabolic processes, such as insulin sensitivity, glucose metabolism and ketogenesis.

Keywords: Lin28, glucose metabolism, ketogenesis

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HLA-Associated Adverse Drug Reactions – a Scoping Review

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Introduction: Pharmacogenetics is an important approach to prevent the occurrence of idiosyncratic adverse drug reactions (ADRs) associated with the human leukocyte antigen (HLA) system, which is responsible for immune recognition in humans. It has been shown that preemptive pharmacogenetic testing for HLA alleles can help prevent associated ADRs by avoiding exposure in carriers of certain alleles [1].
Aims: To compare the current evidence for HLA-mediated ADRs found in literature to pharmacogenetic information in Swiss drug labels (Schweizer Fachinformation). We aimed to compile literature on HLA-associated ADRs and to identify parameters that determine whether pharmacogenetic information found in the literature is integrated in drug labels.
Methods: We conducted a literature review according to the suggested methods by Preferred Reporting Items for Systematic Reviews and Meta-Analyses-Scoping Review (PRISMA-ScR) [2]. The included studies had to describe an association of any HLA-mediated ADR with any drug available on the Swiss market and to show an association between the genotype and the occurrence of ADRs. Only primary literature in English and German from the year 2002 onwards was included.
Results: A total of 136 studies were included in the qualitative synthesis of the Scoping Review. Most of the included studies were retrospective while one was a prospective randomized controlled trial (RCT). The association of carbamazepine-induced cutaneous drug reactions to HLA-B*1502 was found most frequently. The literature for abacavir, allopurinol, carbamazepine, oxcarbazepine, and phenytoin clearly pointed out responsible alleles and the drug label adopted this information. The evidence of HLA-associated ADRs was inconsistent for lapatinib, pazopanib, and flucloxacillin, however the corresponding drug labels mention the involved alleles. Finally, no shared parameters could be identified, that would allow to predict whether pharmacogenetic information should be included in the drug labels.
Conclusions: The Scoping Review revealed that the field of HLA-mediated ADRs lacks structure in the identified studies and that a large amount of heterogeneity is present in the results. Both findings have an impact on the clinical validity as well as the clinical utility of the found associations. A standardized procedure for translating the current knowledge about pharmacogenetic associations into the drug labels is needed in order to provide consistent information on PGx to support health care professionals.

Keywords: pharmacogenetics, human leukocyte antigen, adverse drug reactions, drug label, PRISMA-Scoping Review

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Formulation and Characterization of Cyclic Dinucleotide-Polyethylenimine Nanoparticles

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Introduction: Hepatocellular carcinoma (HCC), the most frequent solid tumor of the liver, has a very poor prognosis, being the second most common cause of death from cancer worldwide. Despite the increasing knowledge on the molecular mechanisms underlying hepatic carcinogenesis, effective therapeutic strategies are still an unmet clinical need. To maximize site-selectivity and therapeutic efficacy reducing systemic side effects, drug delivery systems (beads) may be applied locally through transarterial chemoembolization (TACE), and may be loaded with chemotherapeutic drugs or immunostimulants. In our case, the cyclic dinucleotide (CDN) 2`3`cGAMP was used. CDNs activate the stimulator of interferon genes (STING) pathway that plays an important role in the activation of type I interferons in antigen presenting cells (APCs) in response to cytosolic nucleic acid ligands.
Methods: Formulation of nanoparticles by the ionic gelation method of linear polyethylene imine (PEI; 4 and 25 kDa) with 2`3`cGAMP and tripolyphosphate anions (TPP), as a common cross-linker was performed. Optimization of parameters such as PEI/2`3`cGAMP/TPP ratio, concentration, and polymer molecular weight on the particle size distribution and stability were investigated. Particle size, zeta potential (ZP) and size distribution (PDI) of PEI/2`3`cGAMP/TPP complexes were determined by dynamic light scattering (DLS). In addition, nanoparticles were investigated by electron microscopy (SEM) and their cytotoxicity measured by WST1 assay on murine macrophage-like RAW cells. Experiments were performed at two polymer concentrations (0.1 and 0.01 mg/mL). Ratio of PEI cation: 2`3`cGAMP anion:TPP anion is presented as the molar ratio of PEI nitrogen to 2`3cGAMP and TPP phosphate.
Results: Based on DLS, values for z average size were significantly higher ($P<0.05$) at lower concentrations of polymer. In contrast, there was no significant difference at the same lower concentration in between two different molecular weights of PEI (4 and 25 kDa). Nevertheless, we could see a trend in decreasing zeta potential of 25 kDa compared to 4 kDa. The high PDI values remained the same in all cases, while SEM results were suggesting the most homogeneous size distribution in case of PEI 25 kDa. Even though it was previously reported that the increase of PEI molecular weight increases its cytotoxicity, in our work we did not see differences in between the two polymers of different molecular weight.
Conclusion: We managed to formulate optimized nanoparticles with higher stability (zeta potential) and acceptable toxicity at low concentration using 25 kDa PEI.

Keywords: STING pathway, polyethylenimine, liver cancer, nanoparticles

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Development of a Salmonella Infection Model in Zebrafish Embryos

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Introduction: Infections caused by Salmonella cost millions of lives across the world every year [1]. Whereas systemic infections can usually be controlled using standard antibiotics, complete eradication is often difficult since Salmonella are able to reside within immune cells such as macrophages. These macrophages can act as a reservoir for salmonella from which they can escape and reinitiate the infection after antibiotics treatment is terminated [2]. Moreover, several antibiotics (e.g. tobramycin) show poor uptake into macrophages [3]. To this end, novel antibiotics formulation approaches (e.g. nanoparticles) are required in order to reach and eradicate Salmonella persistent in macrophages.
Aim: In this study, we developed an *in vivo* Salmonella infection model using zebrafish embryos.
Methods: We injected varying amounts of Salmonella at different zebrafish sites and assessed Salmonella biodistribution (i.e. uptake into macrophages) and survival of infected zebrafish embryos.
Results: We developed a Salmonella infection model in zebrafish embryos and treated the infected zebrafish embryos with newly designed antibiotics-loaded nanoparticles (i.e. liposomes) that are being taken up by macrophages. Treatment of zebrafish embryos with antibiotics-loaded nanoparticles resulted in an increased survival rate.
Conclusion: We successfully implemented a Salmonella infection model in zebrafish opening the opportunity to determine the *in vivo* efficacy of antibiotics-loaded nanoparticles and free antibiotics. This infection model is currently used in a research project aiming to deliver tobramycin to infected macrophages within zebrafish embryos.

Keywords: nanoparticles, targeted drug delivery, antibiotics, infectious diseases, animal model development

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Interaction of Tranquilizing Herbs with the Drug Transporters OATP2B1 and OATP1A2

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Introduction: Herbal medications used in the treatment of sleep disorder and anxiety often contain extracts of *Valeriana officinalis* or *Passiflora incarnata*. Valerenic acid in *V. officinalis* and orientin as well as apigenin with its glycosylated form vitexin in *P. incarnata* are thought to contribute to their therapeutic effect. Due to their chemical properties it is assumed that these molecules rely on an active transport across cellular membranes. In terms of cellular uptake the ubiquitously expressed organic anion transporting polypeptide (OATP)2B1 and the OATP1A2, which is highly expressed in the brain, may play a role.
Aims: It was the aim of this study to test, whether the selected constituents of the herbal remedies are interacting with OATP2B1 or OATP1A2.
Methods: MDCKII cells overexpressing OATP2B1 or OATP1A2 were used to determine the influence of valerenic acid, apigenin, orientin or vitexin on the cellular accumulation of estrone 3-sulfate (E_1S), a known substrate of both transporters. Subsequently, competitive counterflow experiments were applied to test whether identified inhibitors are also substrates of the transporters. In a next step commercially available *V. officinalis* and *P. incarnata* preparations were assessed for interaction with the transporters and screened for presence of the selected constituents by applying high performance liquid chromatography (HPLC).
Results: Valerenic acid was interacting with neither OATP2B1 nor OATP1A2, however, OATP2B1 and OATP1A2 mediated transport of E_1S was inhibited by the tested constituents of *P. incarnata* except of vitexin which didn't show interaction with OATP1A2. Competitive counterflow revealed that orientin is a substrate of both transporters whereas apigenin was only transported by OATP1A2 and vitexin only by OATP2B1. A commercially available *P. incarnata* preparation was interacting with both OATPs and with HPLC vitexin and orientin were identified as highly abundant flavones.
Conclusions: In conclusion, our data indicate that constituents of *P. incarnata* extracts may alter the transport function of OATP2B1 and OATP1A2, which could impact the uptake of other drugs or endogenous substrates leading to drug-herb interactions. Nevertheless, further studies are needed to identify the constituents in commercially available *P. incarnata* formulations which are involved in the interaction.

Keywords: *Passiflora incarnata*, *Valeriana officinalis*, OATP2B1, OATP1A2, drug-herb interaction

Combination of Albumin-Binding ^{177}Lu-PSMA-ALB-56 and Fast-Cleared PSMA-Inhibitors: Optimization of the Pharmacokinetics

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Introduction: Prostate-specific membrane antigen (PSMA)-targeted radionuclide therapy of metastatic castration-resistant prostate cancer (mCRPC) showed promising clinical results. In order to optimize the dose to the tumor and, therewith, improve the effect of the treatment, we have recently designed an albumin-binding PSMA radioligand (^{177}Lu-PSMA-ALB-56 [1]) with enhanced circulation time. The (pre)clinical evaluation revealed remarkable increase in tumor uptake and therapeutic efficacy. ^{177}Lu-PSMA-ALB-56 showed, however, also increased renal retention at early time-points (1 and 4 h after injection), potentially leading to radionephrotoxicity.
Aims: The aim of this study was to reduce the increased kidney uptake of ^{177}Lu-PSMA-ALB-56 at early time-points to further improve this concept, by means of a simultaneous injection of a fast-cleared PSMA inhibitor.
Methods: To investigate the approach, Single Photon Emission Computed Tomography **(**SPECT) dual imaging studies were performed by application of ^{177}Lu-PSMA-ALB-56 mixed with a 2.5-fold molar excess of the fast-cleared PSMA inhibitor ^{67}Ga-PSMA-11 and injected into PSMA-positive tumor-bearing mice. Scans were acquired 1, 4 and 24 h after the injection and the images reconstructed using the γ-energies of ^{177}Lu and/or ^{67}Ga. Biodistribution studies were carried out in the same tumor mouse model after co-injection of ^{177}Lu-PSMA-ALB-56 with a 2.5-, 5- or 10-fold molar excess of 3 different inhibitors (PSMA-11, 2-PMPA and ZJ43). Selected tissues were collected at 1, 4 and 24 h p.i. for counting and the results were expressed as injected activity per gram of tissue mass (% IA/g). The area under the curve for the first 24 h p.i. (AUC_{24h}) was determined for the uptake of ^{177}Lu-PSMA-ALB-56 in PSMA-positive tumors and kidneys based on non-decay-corrected biodistribution data.
Results: Preliminary dual imaging studies showed that the co-injection of ^{67}Ga-PSMA-11 reduced the kidney uptake of ^{177}Lu-PSMA-ALB-56 at 1 and 4 h p.i. The uptake in the tumor was only slightly reduced at these time points, but no clear reduction was visible at the 24 h time-point. SPECT images of the ^{67}Ga-PSMA-11 confirmed the anticipated high renal accumulation at early time points after injection. Biodistribution studies showed that the co-injection of a PSMA inhibitor, irrespective of the molar excess used, effectively reduced the kidney uptake of ^{177}Lu-PSMA-ALB-56 at early time points (up to 80%). As a consequence, the AUC_{24h} for kidney uptake was reduced by ~30%. As anticipated from the imaging studies, the tumor uptake (AUC_{24h}) was reduced by ~25% when PSMA-11 and ZJ43 were used as inhibitors. The co-injection of 2-PMPA, on the contrary, caused no significant ($p>0.05$) reduction in the tumor AUC_{24h}.
Conclusions: The injection of a low molecular-weight PSMA inhibitor, improves the pharmacokinetics of ^{177}Lu-PSMA-ALB-56 by effectively reducing its kidney uptake while only slightly affecting the tumor accumulation. Considering the interest in albumin-binding PSMA radioligands, we believe that this approach could have an impact on clinical practice in future and be extended to other radioligands with similar pharmacokinetic properties.

Keywords: prostate cancer, PSMA, albumin-binding PSMA, dual imaging, SPECT imaging

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High Affinity hERG and Low Affinity Cav1.2 Blockers Dehydroevodiamine and Hortiamine in Decoctions of the TCM drug Evodiae fructus

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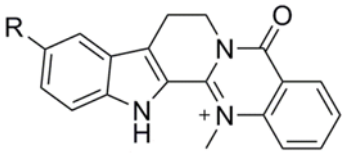
Introduction: Most herbal drugs used in Traditional Chinese Medicine (TCM) are considered safe based on their use over centuries. However, the major alkaloids dehydroevodiamine (Fig. 1: **1**) and hortiamine (**2**) in Evodiae fructus (dried fruits of *Evodia rutaecarpa*) were recently found to be potent blockers of the hERG (human Ether-a-go-go Gene) channel [1]. hERG channel inhibition in combination with low inhibition of the $Ca_V1.2$ channel is associated with proarrhythmic effects, which we confirmed *in vitro* and *in vivo* (rabbit and cAVB dog models). The herbal drug and *Evodia*-containing products are freely available via suppliers of TCM drugs and via internet vendors.
Aims: Is there a cardiac risk associated with the use of Evodiae fructus decoctions in TCM?
Methods: Aqueous decoctions were prepared as commonly used in TCM from different commercially available products and were subsequently lyophilized [2]. The alkaloid contents were determined via HPLC-DAD and the decoctions were tested for inhibition of hERG channels stably expressed in HEK 293 cells. Currents on the HEK 293 cells were studied via the planar patch clamp technique.
Results: Approximately ¼ of the alkaloids present in the drug were extracted into the decoctions. The recommended daily dose (RDD) of Evodiae fructus is 1.5 - 4.5 g/d. This corresponds to the ingestion of significant amounts of the investigated alkaloids (1.0 - 12.3 mg/d of compound **1** and 0.12 – 1.85 mg/d of compound **2**). *In vitro* IC_{50} values for hERG current inhibition were between 2.7 and 8.5 µL of the decoctions (total amount consumed per day: 150-450 mL). A low inhibition of Cav1.2 (IC_{50} of I_{Ca} inhibition >50 µM) channels by the decoctions was found.



1 R = H
2 R = OCH_3

Figure 1 Structures of dehydroevodiamine (**1**) and hortiamine (**2**)

Conclusions: Decoctions of Evodiae fructus lead to the intake of significant amounts of alkaloids **1** and **2**. This hints at–a potential cardiac liability for Evodia decoctions, especially because the comparably low potency inhibition of Cav1.2 suggests a high risk for pro-arrhythmic effects [3].

Keywords: cardiac liability, hERG channel, alkaloids, *Evodia rutaecarpa*

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Amphiphilic Cyclodextrin Particles as a New Generation Vaccine Platform

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Introduction: Natural cyclodextrins (CDs) hold great potential as a novel vaccine delivery platform. CDs are well established as pharmaceutical excipients for increasing drug solubility, stability, and affording prolonged drug release. Derivatisation can render CDs amphiphilic enabling them to self-assemble into nanoparticles and entrap or adsorb antigens in order to deliver them to local immune cells at the injection site. Supramolecular CD-structures have already been described for gene and drug delivery [1], however, to our knowledge no report of a CD-based particulate vaccine delivery system has appeared in the literature to date.
Aims: We aim at developing amphiphilic β-CD-based nanoparticles as vaccine delivery system for antigens to promote their efficient uptake by antigen presenting cells (APCs) and elicit strong immune responses.
Methods: In a first step, amphiphilic β-CDs were synthesized by grafting thioalkyl chains of different lengths (C4, C6, C8, C12, C18) onto the primary face of natural β-CDs [2]. Different particle formation methods were evaluated for their suitability to form nanoparticles (e.g. vesicle formation via extrusion, sonication, nanoprecipitation). As nanoprecipitation [3] showed the most promising results, the process was optimized. The obtained particles were characterized by their size distribution, zeta-potential, and dye entrapment capacity. Nanoparticles (NP) of 3 different amphiphilic β-CD derivatives (NPC4, NPC8, NPC12) were compared and their morphology was assessed using transmission and cryo-scanning electron microscopy (TEM and cryo-SEM). For encapsulation studies, lipophilic dye as well as MHC class I restricted model peptides were used. First *in vitro* studies were carried out with bone marrow derived dendritic cells (BMDCs) and IC-21 macrophages.
Results: Butylthio-, hexanethio-, octanethio-, dodecanethio-, and octadecanethio-β-CDs were produced and isolated in high yield (60-70%). The nanoprecipitation method reproducibly formed amphiphilic β-CD nanoparticles using the different derivatives NPC4, NPC8, and NPC12, displaying a hydrodynamic radius of around 150-200 nm with a narrow size distribution. TEM and cryo-SEM imaging visualized spherical shapes and confirmed the results obtained by dynamic light scattering. The nanoparticles did not produce significant toxicity on BMDCs and IC-21. Antigen entrapment was achieved with LCMV gp33 peptide, ovalbumin 257-264 peptide, and fluorescein labelled ovalbumin.
Conclusions: We developed nanoparticles from different amphiphilic β-CD derivatives to obtain a novel platform for vaccine delivery. The platform provides the capacity to deliver hydrophilic as well as hydrophobic antigens. *In vitro* studies showed that nanoparticles were actively taken up by APCs. These findings underline the potential of amphiphilic CD nanoparticles as vaccine delivery system, and further *in vitro* studies are ongoing. The future objective will be to enhance the uptake of peptide antigens entrapped in the nanoparticles by murine and human APCs for subsequent presentation and activation of CD8+ T-cells. The most promising candidates will be tested *in vivo* for their potential to elicit antigen specific immune response.

Keywords: amphiphilic cyclodextrins, vaccines, nanoparticles, antigen delivery

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Stabilizing Polysorbate 20 and 80 Against Oxidative Degradation

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Introduction: Surfactants, e.g. polysorbate 20 (PS20) and polysorbate 80 (PS80), are used in biopharmaceutical formulations, among other things, to stabilize therapeutic proteins against interfacial stress. PS20 and PS80 - both containing ester and ether linkages - are prone to hydrolytic and oxidative degradation. It has been shown that the oxidative degradation pathway is relevant under pharmaceutical conditions [1].
Aims: The purpose of this study was to investigate if oxidation of PS20 and PS80 in aqueous solution can be prevented by the addition of the antioxidants butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA).
Methods: Therefore, samples (aqueous PS20 and PS80 samples containing either BHT, BHA or no stabilizer) were incubated at elevated temperature (40°C, 75% rH) with air exposure for 7 weeks. Afterwards, the following analyses were performed: HPLC fluorescence micelle assay (FMA) to determine remaining concentration of intact (i.e. micelle-forming) polysorbate, ferrous oxidation - xylenol orange (FOX) assay to quantify peroxide level, pH measurement, HS-GC-MS to characterize volatile degradants, and LC-UV-MS to determine formation of free fatty acids.
Results: Samples containing either BHT or BHA were better stabilized in both aqueous PS20 and PS80 solutions compared to non-stabilized samples, as seen by higher micelle concentration, lower peroxide levels, stable pH, and lower degree of volatile degradants. The antioxidant levels in the stressed samples were decreased, indicating that indeed the antioxidants were degraded instead of the polysorbates.
Conclusions: It can be concluded that aqueous PS20 and PS80 solutions containing BHT and BHA are more stable, i.e. less susceptible to oxidative degradation, compared to non-stabilized samples.

Keywords: polysorbate, oxidation, degradation, BHT, BHA

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P-40

Functionalized Calcium Carbonate Microparticles as a Carrier for Lipid-based Oral Formulations

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Introduction: Lipid-based drug delivery systems have great potential for enhanced dissolution and oral bioavailability for low soluble drugs (BCS Class II). However, production of solid dosage forms from lipids is difficult and costly. Loading of lipid-based formulations into a porous drug carrier can help with dissolution enhancement, transform them into free flowing powders and facilitate further processing [1].
Aims: The aim of our study is to demonstrate a new method for material distribution analysis into porous microparticle carriers, such as functionalized calcium carbonate (FCC) [2], and characterization of this formulation as a solid lipid-based oral drug delivery system.
Methods: Nifedipine as a model low-soluble drug and DMPC and DPPC as model phospholipids were used in this study. Loading efficiency and material distribution in the internal structure of FCC microparticles were investigated using focused ion beam scanning electron microscopy (FIB-SEM) and mercury intrusion porosimetry (MIP). The formulations were characterized regarding drug loading, hydration and *in vitro* dissolution in simulated gastric fluid.
Results: The results of FIB-SEM analysis revealed the limitations of other common methods for study of pore filling. We showed that DPPC as the model phospholipid is loaded deep into the porous structure of the carrier [3]. The results indicated that loading of DMPC and nifedipine in FCC obtained a total loading ratio of 40% *w/w*. Upon exposure to simulated gastric fluid, FCC was dissolved and the phospholipids were released in form of multi-lamellar vesicles. The results of *in vitro* dissolution in simulated gastric fluid corroborated the FIB-SEM predictions of enhanced dissolution rates compared to unloaded mixture of the components.
Conclusions: The obtained results suggest that lipid-based FCC solid formulations can serve as a strategy for dissolution enhancement of low water-soluble drugs.

Keywords: phospholipids, porous microparticles, dissolution enhancement, solid dosage forms

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Following the Nature of Dicer Cleavage Under Influence of RNA Binding Proteins

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Introduction: MicroRNAs (miRNAs) are short, non-coding RNAs with a broad impact in biological mechanisms [1]. During miRNA biogenesis a main step is cleavage of the terminal loop of precursor miRNAs (pre-miRNA) by endonuclease III Dicer resulting in a miRNA duplex, consisting of a 5p and 3p strand. For some miRNAs only one of those strands is processed to the dominant guide miRNA (e.g. miR-107-3p), whereas for other miRNAs both strands can be active (e.g. miR-20b). Each miRNA is capable of regulating multiple genes by translation repression or mRNA degradation, and then again each gene can be controlled by multiple different miRNAs, hence small changes in miRNA expression levels can result in big phenotypic effects, e.g. in development or diseases [2]. RNA-binding proteins (RBP) are prominent regulators of miRNA processing and therefore hold great spatio-temporal regulatory functions.
Aims: In this study we demonstrate the binding of a RBP family of Rbfox to sequence-specific motifs in the terminal loop of pre-miRNAs and how this interaction further influences levels of mature miRNAs.
Methods: UV-Crosslinking and Surface-Plasmon-Resonance (SPR) was performed in order to confirm the interaction of Rbfox RNA recognition motifs (RRM) to specific sequences in the terminal loop region of pre-miRNAs. A newly described [^{32}P]-labeling technique was used to investigate 5p- and 3p-miRNA *in vitro* Dicer processing in presence of the RBP Rbfox [3]. Denaturing gel electrophoresis allowed sample degradation and size separation.
Results: The RRM of Rbfox proteins binds sequence specifically to a motif present in terminal loop regions of precursor miRNAs. This interaction was abrogated when one nucleotide of the motif was mutated. Furthermore, *in vitro* Dicer assays show a down-regulated miRNA processing in conditions where Rbfox RRM is present.
Conclusions: Consequently, Rbfox proteins have a regulatory function on pre-miRNAs containing specific sequences in their terminal loop region and thereby Rbfox proteins might be able to influence developmental stages or disease conditions.

Keywords: microRNA, Dicer processing, RNA-binding proteins, Rbfox

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Fabrication of Personalized Bioresorbable Airway Stents by 3D Printing

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Introduction: Airway stents are used to restore the airway patency in patients suffering from central airway obstruction (CAO). A number of conditions can cause CAO, such as prolonged intubation-related injury, congenital malformations, and tumors, all of which are characterized by decreased quality of life with potentially fatal outcome [1]. State-of-the-art stents are made of biocompatible silicone elastomer by injection molding. However, they come in a very limited number of shapes and sizes, while every personalization is expensive and time-consuming. In addition, they exhibit propensity to migrate and usually require a subsequent extraction [1]. These challenges could be overcome by 3D printing of personalized bioresorbable airway stents based on computed tomography (CT) scans of individual patients. As a 3D printing technique characterized with high resolution, good surface quality, desktop size, and relatively low cost, digital light processing (DLP) is endowed with great potential for this type of manufacturing [2]. However, its biomedical application is restricted by the lack of «pharmaceutical inks» [3].
Aims: We aim to develop personalized bioresorbable airway stents by DLP 3D printing of biodegradable biocompatible polymeric materials.
Methods: After the initial screening of polymeric materials, two series of biodegradable polymers were synthesized from D,L-lactide and ε-caprolactone with varied molecular weights and topological structures. Their composites with different feeding ratios were used for 3D printing in a customized DLP printer. The mechanical properties of the printed specimens were evaluated in tensile and compression tests. Optimized materials were further tested for degradation and *in vitro* cytotoxicity. Degradation study was performed in a buffer at pH 7.4 at 37 °C for 20 weeks, and the loss of mass and compressive strength of the printed stents over time was investigated. Cytotoxicity of the printed specimens was assessed in A549 cell line using the MTS colorimetric assay. A customized tracheal stent was printed by DLP in combination with CT scans of a New Zealand white rabbit. Radiopaque stents were also made and visualized by X-rays in a rabbit cadaver.
Results: The polymeric materials exhibited multi-tunable mechanical properties. The optimized materials showed compressive strength comparable to that of a silicone elastomer, with the additional ability to degrade under physiological conditions. The 3D printed tubular specimens exhibited slow mass decrease within 20 weeks, while maintaining their mechanical properties for at least 8 weeks. The printed specimens demonstrated good cytocompatibility, with cell viability above 95%. A customized 3D printed radiopaque CT-based tracheal stent was successfully inserted and visualized in the trachea of a rabbit cadaver.
Conclusions: We developed new biodegradable and biocompatible polymeric materials as «pharmaceutical inks» suitable for DLP and successfully applied them to produce customized radiopaque tracheal stents with mechanical properties comparable to that of the state-of-the-art stents.

Keywords: 3D printing, airway stents, biodegradable polymers, central airway obstruction (CAO), digital light processing (DLP)

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P-43

Triblock Copolymeric Micelles Based on Polylysine for siRNA Delivery

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Introduction: Failure to inhibit STAT3 expression by oligonucleotide based therapeutics resulted in the development of a novel nanoplatform dedicated to siRNA delivery. The success mainly depends on the carrier for effective gene transfer. A non-viral vector based on amphiphilic triblock co-polymers was conceived as a biocompatible siRNA carrier. The structure of the oligocation (Poly-L-Lysine, 10 units 1.3kDa) was selected based on computational tuning of binding mechanism to an siRNA against human STAT3 [1]. The vector was consecutively synthesized. *In vitro* cytotoxic assays previously demonstrated that the novel carrier is non-toxic on lung carcinoma cells (A549).
Aim: The aim of this work was to synthesize, characterize and evaluate the safety and efficacy of a novel polymeric platform.
Methods: Triblock copolymers were synthesized and characterized by ^{1}H-NMR and MALDI-TOF analysis. Micelles were prepared by sonication and solvent evaporation technique. The critical micellar concentration (CMC) was evaluated by fluorescence measurements using Nile Red. Characterization of the size, zeta potential and molecular weight of the carrier was performed using a Malvern Zetasizer at 25°C. The size of the vector was also observed by transmission electron microscopy (TEM). A capillary electrophoresis (CE) method enabled the quantification of siRNA complexed within polymeric micelles. EC50 in A549 was assessed by WST-1 assay after exposure to micelles containing STAT3-siRNA. An immunoblotting technique has been conducted in identifying the protein of interest recognized by anti-STAT3 antibodies.
Results: The dispersity is monomodal and the size obtained by measuring intensity of scattered light was correlated in number by TEM with an average of 90 nm in diameter and zeta potential of approximately 45 mV. A molecular weight of 4830 kDa were observed by static light scattering resulting in 9000 cationic charges per micelles. The micelles are stable in a diluted concentration with a critical micellar concentration of 100 mg/L. *In vitro* cytotoxic assays demonstrated that the novel triblock co-polymer is biocompatible and non-toxic on cells. More than 80 % of siRNA is complexed at N/P 1 with a detection of siRNA in less than 5 min. First experiment with one N/P ratio did not show efficacy on proliferation of lung carcinoma cells. Changes in the expression of STAT3 are currently insufficient to demonstrate an efficacy.
Conclusions: The amphiphilic triblock co-polymers were successfully synthesized, self-assembled into cationic polymeric micelles, characterized and the safety of the new biomaterial was demonstrated *in vitro*. Optimization of N/P ratio and formulation technique are under investigation to demonstrate a gene silencing efficacy. Uptake studies with fluorescently-labeled siRNA and RT-qPCR method will be further investigated.

Keywords: polymeric micelles, siRNA delivery

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Development of Hydrophobic Salts of *Myo*-inositol Hexakisphosphate

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Introduction: *Myo*-inositol hexakisphosphate (IP6) is a natural occurring molecule with high calcium chelating ability and a novel drug candidate developed to treat cardiovascular calcification disorders, for which yet no approved therapy exists. The half-life after subcutaneous bolus injection of 10 mg/kg (efficacious dose in murine calcification model) was 3.9 min in rats, thus complicating its development as a chronic ambulant therapy [1].
Aims: Development of hydrophobic salts to facilitate a sustained release application, in order to prolong the half-life of IP6 following extravascular parenteral administration.
Methods: Different solvent systems and various cationic counterions used in different molar ratios were employed for hydrophobic salt formation of IP6. The identity of the obtained salts was confirmed by structural analysis (^{1}H-nuclear magnetic resonance (NMR) and ^{31}P-NMR spectroscopies) and their physicochemical properties were characterized by melting temperature determination, bright field and polarized light microscopies, and X-ray diffraction (XRD). Aqueous solubility was assessed by overnight equilibration and compound quantification was performed by HPLC-ELSD. Cell culture experiments were performed with human THP-1 monocytes assessing cell viability and TNF-α release after 24 h-treatment with selected salts.
Results: Three methods for salt formation were developed: evaporative crystallization using acetonitrile/water, cooling crystallization with ethanol, and precipitation in DMSO/water. A total of 14 different IP6 salts were produced. Structural analysis was performed on these salts confirming successful salt formation at various IP6 to counterion ratios. Compared to the control sodium salt of IP6, aqueous solubilities could be decreased by up to 1000 fold. *In vitro* cell viability of THP-1 monocytes was, however, reduced by 50% for the investigated IP6 salts. None of these salts significantly increased TNF-α levels in cell culture supernatants.
Conclusions: In conclusion, we successfully developed 3 methods for preparing salts of IP6 displaying various levels of hydrophobicity. The method comprising acetonitrile/water was found to be the most suitable solvent system for salt formation. The herein presented results must be confirmed in follow up experiments and future studies must be conducted to identify conditions of crystallization and source of toxicity.

Keywords: hydrophobic salt, sustained release, preformulation

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Splice-Switching Oligonucleotides for Erythropoietic Protoporphyria, a Blood Genetic Disease

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Introduction: Erythropoeitic protoporphyria (EPP) is a genetic disorder affecting in average 1/100.000 individuals. Patients suffer from painful photosensitivity upon exposure to the blue component of light, coming from natural and artificial sources. EPP is caused by the deficiency in ferrochelatase (FECH) protein, the last enzyme of heme biosynthesis pathway, and the uncontrolled accumulation of its substrate protoporphyrin IX (PPIX) in red blood cells and in the skin vessels. EPP onset is quite peculiar, as it requires a combination of two independent genetic events on both alleles of the ferrochelatase (*FECH*) gene, both reducing the production of FECH protein. In one allele, a non-sense or missense mutation shuts the synthesis of the functional protein down. In the other, an intronic single nucleotide polymorphism (SNP) switches FECH messenger RNA (mRNA) sequence from the functional one to an aberrant one, which is directed by the cell machinery to degradation. Steric-blocking oligonucleotides are a clinically validated class of oligonucleotide drugs and are designed to directly bind the precursor messenger RNA to modulate its mature sequence. Such a molecule is used here to reverse the impact of the SNP on FECH mRNA, forcing the cell machinery to produce again the functional protein. A critical challenge for the project is to achieve oligonucleotide delivery *in vivo* in the bone marrow to maturating red blood cells. Besides, in order to alleviate light photosensitivity symptoms, oligonucleotide effects on FECH and on its substrate PPIX need to be obtained before mature blood cells get released into the bloodstream.
Aims: (i) Screen and select a lead single-stranded oligonucleotide, capable of restoring correct FECH mRNA production *in vitro*; (ii) identify moieties to be covalently attached to the lead oligonucleotide for improved bone marrow delivery, and establish reliable synthesis routes to access such conjugates; (iii) produce cherry-picked conjugates on large scale for *in* vivo injection in a first-generation mouse model; investigate uptake in key tissues, biological activity on mRNA levels, and metabolism profile; (iv) develop an EPP transgenic mouse model for phenotypical studies.
Methods: Aim (i) Oligonucleotide synthesis and screening; cellular splicing assay. Aim (ii) Oligonucleotide bioconjugation to cell penetrating peptides and lipids; synthesis methodology. Aim (iii) Quantification of oligonucleotides in tissues by CL-qPCR; quantification of FECH correct mRNA levels in tissues by RT-qPCR; metabolism endpoint analysis of oligonucleotide conjugates. Aim (iv) CRISPR-Cas9 genome editing system.
Results: Aim (i) A 22-nucleotide oligonucleotide was selected after screening and able to fully correct FECH aberrant splicing at 10 nM concentration in a model minigene system. Aim (ii) A library of 22 peptide- and lipid- conjugates was produced, from which 3 conjugates were selected for *in vivo* experiments. Aim (iii) The unconjugated lead and the 3 conjugated versions were injected for 2 weeks s.c. at 50 mg/kg in our mouse model. One conjugate was shown to be active in the bone marrow, where it generated an 80% increase of FECH correct mRNA levels. Aim (iv) Work is in progress.
Conclusionss: We have identified a lead oligonucleotide which corrects FECH mRNA *in vitro*. Conjugation of this lead structure to a cholesterol moiety led *in vivo* to biological activity in bone marrow, a key tissue in EPP, without toxicity alert. As our first-generation mouse model only provides a molecular readout, the laboratory is currently working on the generation of a transgenic mouse model. It will open the way for phenotypical studies on light photosensitivity, the main life-quality impacting symptom in patients.

Keywords: genetic disease, oligonucleotide drug, bioconjugation, tissue-enhanced delivery

Highly Engineered Antibacterial Peptide Dendrimers Coupled to Chitosan Derivatives to Efficiently Eradicate *Pseudomonas aeruginosa*

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Introduction: Antimicrobial resistance (AMR) is a global healthcare concern that continues to worsen despite the efforts in finding solutions. It is estimated that by the end of 2050, AMR could lead to more than 10 million deaths annually, more than 8.2 million that currently die from cancer [1]. One of the most challenging MDR bacteria is *Pseudomonas aeruginosa*, which is part of the problematic collection called ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* species). The drug pipeline is a cause for alarm as most of the big pharma industries do not focus on this issue. Therefore, there is an urgent need to develop new strategies to deliver these AMPs and AMPDs avoiding their degradation, still keeping a low toxicity at therapeutic concentrations. Several peptides, known as antimicrobial peptides (AMPs) and AMP dendrimers (AMPD), hold great potential against microbial infections. However, their clinical application is hindered by different challenges, including toxicity, low selectivity, fast degradation and short half-life [2]. Covalent coupling of AMPs to chitosan was shown to be active against *Pseudomonas aeruginosa*, one of the most challenging multidrug- resistant bacteria in chronic wounds, such as burns, diabetic and non-healing wounds that favor the formation of a biofilm on top of the wound [3].
Aims: To tackle these drawbacks, we have developed an unique chemical technology based on coupling AMPDs to chitosan derivatives, such as CMTMC (O-carboxymethyl-N,N,N-trimethyl-chitosan). Moreover, we have developed different strategies for topical delivery to the patient, using nanoparticles, gels and foams.
Methods: AMPDs were covalently coupled to chitosan through a controlled chemistry. Polyelectrolyte formulations, such as nanoparticles (NPs), hydrogels and foams for topical administration were obtained. NPs were analyzed for their size, charge and stability in different media over time by dynamic light scattering (DLS). Foams were obtained upon hydrogel lyophilization and were analyzed by scanning electron microscopy (SEM).
Results: The degree of conjugation of AMP to chitosan derivatives was determined to be ± 30% for all the conjugates using amino acid analysis. The results showed that covalent immobilization of AMPD to chitosan derivatives inhibited the growth of *P. aeruginosa* with a MIC (minimal inhibitory concentration) of 2-16 µg/mL. With this technology, we obtained a very strong synergy in terms of antimicrobial efficacy and very low toxicity to human fibroblasts at high AMPD concentrations (10-fold MIC after 2 days of exposure). Interestingly, the antimicrobial activity of the incorporated AMPD any of the developed formulations was preserved towards *P. aeruginosa*, without toxicity to human cells.
Conclusions: Covalent immobilization of AMPDs to chitosan overcomes the drawbacks and limitations of the AMPDs alone. Nanoparticles and foams can be developed for accommodation of the AMPs within these nanostructures to be released at needed time against bacteria. This technology is very unique compared to conventional techniques for covalent coupling of peptides to different materials, which resulted in very low and uncontrolled degree of grafting.

Keywords: antimicrobial resistance, ESKAPE bacterial collection, AMPD, topical formulations

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New Insights Into the Character of Cross-Linking of the miR-CLIP Probes

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Introduction: MicroRNAs (miRNAs) constitute a class of small, endogenous, noncoding RNAs (ncRNAs) and have a great influence on various processes within the cell, including cell growth and differentiation [1]. By base-pairing to their specific, partially-complementary sites located predominantly in the 3' untranslated region (3'-UTR) of the target messenger RNAs (mRNAs), miRNAs participate in the post-transcriptional regulation of gene expression. MiRNAs are responsible for controlling the expression of the majority of human protein-coding genes and their dysregulation has been related to many pathological processes and diseases such as cancer. MiRNAs can be considered as either therapeutic agents or therapeutic targets. In order to use miRNAs as therapeutic, in-depth understanding of their mechanisms of action is of great importance. One of the key challenges is the elucidation of miRNAs' targets, together with their sites of canonical and non-canonical interactions. Imperfect pairing between miRNAs and their target mRNAs in animals, as well as high false positive and false negative rates for current prediction algorithms, generate a need for experimental confirmation. For this purpose, miRNA analogues bearing various cross-linkers can be applied. In the presence of cross-linkers a covalent bond between the miRNA and the mRNA target is formed, enabling elucidation and/or validation of the target site. The microRNA cross-linking and immunoprecipitation (miR-CLIP) approach developed in our group allows capturing predicted and unpredicted miRNA targets in cells employing pre-miRNAs site-specifically modified with biotin and trioxsalen (a psoralen derivative) [2]. We have previously shown that the bis-modified miR-106a probe is able to cross-link to complementary regions present in the mRNA targets, but the exact site of cross-linking has not been determined.
Aims: To determine the site of the cross-linking in the probes with a current design in order to generate universal miR-CLIP probes which could be used to capture predicted and unpredicted miRNA targets.
Methods: Oligonucleotide synthesis, *in vitro* cross-linking assay, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS).
Results: Herein, we present the results of the *in vitro* cross-linking assays with a set of synthesized trioxsalen-labelled miRNA analogues. The results suggest that the cross-linking does not occur with a uridine base-paired with the adenosine bearing trioxsalen moiety, and the cross-linking site seems to be shifted two or three base pairs aside from the expected site of interaction. Tested bis-modified miR-CLIP probes were able to capture miRNA targets in cells and the results were reproducible in the *in vitro* cross-linking assay.
Conclusions: These new observations concerning the site of the cross-linking are taken into consideration while preparing optimized miR-CLIP probes for 3p and 5p miRNAs, with the ultimate goal to achieve unbiased miRNA-mRNA cross-linking.

Keywords: miRNAs, cross-linking, miR-CLIP

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A New Chalcone Derivative with High Pro-Apoptotic Effect on Cisplatin-Resistant Bladder Carcinoma

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Introduction: Despite being the tenth most common cancer worldwide, minimal progress has been made in the treatment of bladder cancer in over two decades. Adjuvant chemotherapy is widely used after transurethral resection, to prevent recurrence and progression of the disease. However, insufficient efficacy with appearance of chemoresistance counteracts its effects. New therapeutic molecules are needed in order to confront the emerging threat of drug resistance. Another strategy is the enhancement of the therapeutic index of the drug by increasing its stability, solubility and bioavailability, along with site-specific delivery using efficient drug carrier systems.
Aims: In this study, a new chalcone derivative was identified by pharmacomodulation, for its ability to induce a G2 accumulation in the cell cycle and subsequent apoptosis of bladder cancer cells.
Methods: Antiproliferative activity was evaluated on both cisplatin-sensitive (RT112) and -resistant (RT112-cp) human bladder cancer cells and compared to that on TERT-NHU normal human urothelial cells. Then, the FDA-approved methoxy-poly(ethylene glycol-*b*-poly(lactic acid)) (mPEG-PLA) block copolymers were successfully synthesized by ring opening polymerization (ROP) in bulk at 100 °C [1] and later used for the formulation of chalcone derivative-loaded micelles. Generated micelles were fully characterized in terms of critical micelle concentration by incorporating Nile Red, z-average particle size distribution using dynamic light scattering (DLS) and morphological observation using Scanning Electron Microscopy, loading amount and efficiency, *in vitro* release and micelle stability. MTT tetrazolium salt and Annexin V/PI assays were used to evaluate cell proliferation and apoptosis before and after vectorization in order to assess the preservation of the antitumor effect.
Results: Results showed that the new chalcone derivative selectively inhibits proliferation of tumor-derived cells versus normal non-tumor cells. Its IC_{50} was about 1.5 µM (after 48 h of treatment) on both cisplatin-sensitive and -resistant bladder cancer cells at great selectivity (IC_{50} of about 34 µM on normal urothelial cells). Chalcone derivative loaded micelles were prepared using mPEG-PLA copolymers with sizes ranging from 30 to 80 nm. Cytotoxic analysis showed a preservation of the antiproliferative activity after vectorization. Results also revealed that it induces an activation of caspase 3 and cleavage of PARP-1 earlier in the cells resistant to cisplatin than in the sensitive ones, and so a subsequent apoptosis of both bladder cancer cells.
Conclusions: Altogether, these findings suggest that this new chalcone derivative is an attractive pro-apoptotic agent with potential chemotherapeutic value. Its poor aqueous solubility, which constitutes a limitation toward effective clinical applications was addressed by its incorporation into nanoparticle carriers.

Keywords: chalcone, bladder cancer, antitumor activity, pro-apoptotic, polymeric micelles

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Investigation of Alternative Scaffolds for Systemic Delivery of Small Interfering RNAs

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Introduction: Small interfering RNAs (siRNAs) are a promising class of novel RNA-directed therapeutics. While exerting their gene-silencing activity similar to micro RNAs (miRNAs) by recruiting the RNA induced silencing complex (RISC) toward mRNA targets, siRNAs are capable of inducing Argonaute 2 (AGO2) mediated target RNA cleavage. Although highly efficient *in vitro*, systemic delivery of the molecules *in vivo* turned out to be the major hurdle for clinical applications. Patisiran was the first siRNA receiving FDA approval in August 2018 [1], nearly 20 years after the discovery of RNA interference [2]. Current clinically relevant siRNAs depend on lipid nanoparticle formulation or heavy chemical modification involving complex targeting moieties and a variety of non-natural building blocks [3], each posing a specific risk when exposed to the human metabolic machinery.
Aim: Investigation of a full phosphorothioate (PS) backbone as an alternative scaffold for naked delivery of siRNAs (PS siRNAs) without the need for extensive chemical modification.
Methods: Oligonucleotide-synthesis, western blot, reverse transcription quantitative polymerase chain reaction (RT-qPCR), chemical ligation qPCR (CL-qPCR), UV melting studies, northern blot, PNA hybridization assay, serum and tritosome stability assay.
Results: A clean synthesis procedure was established for the production of high purity PS siRNAs. A fully PS modified siRNA targeting the oncoprotein Lin28B showed comparable activity to the parent compound (PO siLin28B) *in vitro*. Nuclease resistance of PS siLin28B, both in serum and rat liver tritosomes, was markedly increased compared to the PO analogue. For the purpose of *in vivo* characterization, a PNA hybridization assay was identified as a suitable method for quantification of PS siLin28B in tissue biopsies and outperformed RT-qPCR, CL-qPCR and northern blot. The results showed that PS siLin28B had a broad biodistribution profile in nude mice.
Conclusions: A PS siRNA targeting Lin28B showed efficient knockdown activity in cell culture along with an increased nuclease resistance and a promising biodistribution profile. We are currently investigating additional naturally occurring chemical modifications to further improve the pharmacokinetic properties of PS siRNAs.

Keywords: RNA therapeutics, delivery, siRNA, RNA interference, phosphorothioate

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Synergistic Multi-Drug Combination Active in the Treatment of Renal Cell Carcinoma

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Introduction: First line clinical treatment of primary and metastatic renal cell carcinoma (RCC) with the targeted agents sunitinib and axitinib is very often of limited success. Intrinsic or induced resistance to this current medication diminishes treatment efficacy and overall treatment outcome.There is therefore unmet need for the establishment of innovative treatment strategies, e.g. well designed and validated combination therapies.
Aims: We selected a set of tyrosine kinase inhibitors (TKI) and histone deacetylase inhibitors (HDACi) to test them in combination, monitoring the effect on the cell metabolic activity in a subtype of human metastatic RCC cells, Caki-1. We wanted to measure, whether an optimized low-dose three-drug combination could exceed or potentiate the efficacy of current first line treatment regimens.
Methods: Following the validated streamlined-Feedback System Control technique for drug optimization we determined a synergistic low-dose three-drug combination composed of axitinib (TKI), panabinostat (HDACi), and vorinostat (HDACi).
Results: This drug combination decreased the cell metabolic activity in Caki-1 cells by over 80% and influenced mitosis causing severe cellular abnormalities and apoptosis induction. Fluorescence staining presented that treatment induces the synthesis of cytoplasm and the formation of stress fibers in Caki-1 cells. Moreover, the cell adhesiveness enhanced, which might interfere with the metastatic capacity of Caki-1 cells.
Conclusions: Our data suggest that our optimized low-dose drug combination is highly effective in *in vitro* settings leading to the transformation of the cellular morphology. Cell-cell and cell-environment interactions increase reverting the invasive and metastatic phenotype of Caki-1 cells.

Keywords: drug combination, histones deacetylase inhibitors, metastatic renal cell carcinoma, tyrosine kinase inhibitors

Optimization of a Personalized Drug Combination for Effective Primary Colorectal Cancer Treatment Using 3D Patient-Derived Spheroids Model

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Introduction: Effective treatment options are still lacking for many cancer types, especially in late stage colorectal cancer, mainly due to intrinsic or acquired drug resistance. Drug combinations may overcome the latter by simultaneous targeting of multiple pathways. The streamlined-Feedback System Control (s-FSC) technology allows for the rapid identification of an experimentally verifiable synergistic optimal drug combination (ODC) with minimal experimental effort. Starting from a pool of 10 targeted compounds, optimized drug therapies were specifically optimized for various cell lines representing different subtypes of a cancer type including colorectal and renal carcinoma. We propose here to develop this technology in personalized screening for ODCs in freshly isolated patient-derived cells obtained from tumor tissues of colorectal carcinoma (CRC). By selecting drugs for which clinical data are already available, a rapid progression to phase I/II clinical trials for the optimized drug mixture is envisioned.
Aim: The primary objective of this project is to identify a 3- or 4- synergistic patient-specific optimized drug combination, using the s-SFC platform on freshly isolated patient-derived samples from late (IV) stage primary colorectal cell carcinoma.
Methods: Using the s-FSC validated platform at the Molecular Pharmacology Group, and information on the single drug action of the different compounds on CRC laboratory cell line SW620 we initiated a screen for optimal combinations of drug mixtures on 3D spheroids for the first time. Initially, dose-response curves were performed for 11 individual drugs in both 2D and 3D spheroids models to define their range of activity. Simultaneously, we performed an optimization of the tumor isolation protocol in patient-derived CRC material in order to maintain cell viability up to 10 days post-isolation.
Results: Freshly isolated cancerous cells were successfully maintained with high viability in culture for over a period of 10 days in our optimized supplemented culture media. Furthermore, results from the first iteration of the 11 drugs screen on SW620 spheroids allowed to eliminate 4 antagonistic/inactive compounds from the initial drug pool and highlighted a potential synergistic 4-drug combination.
Conclusions: We successfully optimized a protocol that allows maintaining high viability of patient-derived cells in an adequate time-period necessary to perform an s-FSC based screen. Iteration 2 and 3 of the s-FSC are to be performed for SW620. The same procedure is to be performed with DLD1, HCT116 and LS174T cell lines.

Keywords: colorectal cancer, patient-derived, 3D cultures, spheroids, drug combinations

Investigation of Segmental Activity of Intestinal MRP3 Drug Transporter Using *Ex Vivo* Porcine Intestine

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Introduction: Orally ingested drugs can cross the intestinal wall by either passive or active transport. The latter is mediated by numerous membrane transporters [1]. Evaluation of their effect on drug absorption at an early stage during the development process could be highly beneficial. We recently reported on how porcine intestine *ex vivo* could be used to model drug absorption in humans [2].
Aims: The aim of the present study was to investigate regioselective activity of the multidrug resistance-associated protein 3 (MRP3, basolateral efflux), a transporter belonging to the ATP binding cassette transporter family, in the small intestine (duodenum/jejunum) and the colon. For this reason, the apparent drug permeability $P_{app,pig}$ of fexofenadine, a known MRP3-substrate [1], was studied in the absence and presence of indomethacin, a known MRP3-inhibitor.
Methods: Porcine intestine was harvested immediately after slaughter and kept viable in an ice-cold Krebs Bicarbonate Ringer buffer (KBR) constantly bubbled with a mixture of 95% O_2 and 5% CO_2. After removal of the tunica muscularis, the intestinal mucosa was inserted in an Ussing chamber system. Compartments were filled with KBR (pH 7.4, 38°C), including fexofenadine (100 µM) with/without indomethacin (100 µM) in the donor compartment. Transepithelial resistance was monitored to report on tissue viability. To keep the intestine viable and for buffer circulation, both compartments were constantly oxygenated. Aliquots (400 µL) were withdrawn from the acceptor compartment every 20 min for a period of 2 h. Fexofenadine concentrations in the samples were determined using a Waters UHPLC-MS/MS system and were used to calculate $P_{app,pig}$ across the different tissues.
Results: In the absence of indomethacin, the $P_{app,pig}$ of fexofenadine across duodenum, jejunum and colon were $(1.68 \pm 0.65) \times 10^{-6}$ cm/s, $(2.11 \pm 0.73) \times 10^{-6}$ cm/s, and $(7.78 \pm 1.74) \times 10^{-6}$ cm/s, respectively. After the addition of the MRP3-inhibitor indomethacin to the donor compartment, the corresponding, significantly decreased values measured across duodenum, jejunum and colon were $(0.28 \pm 0.01) \times 10^{-6}$ cm/s ($p<0.009$), $(0.72 \pm 0.03) \times 10^{-6}$ cm/s ($p<0.001$), and $(5.56 \pm 0.99) \times 10^{-6}$ cm/s ($p<0.04$), respectively. The less significant effect of indomethacin on $P_{app,pig}$ in the colon compared to the small intestine was consistent with the increasing MRP3 concentration reported in the colon *in vivo* in piglets [3].
Conclusions: Segmental MRP3-activity of *ex vivo* porcine intestine was successfully demonstrated by evaluating $P_{app,\ pig}$ in absence/presence of indomethacin. Further studies have to be performed to verify: (a) correlation between the segmental transporter concentration and the strength of inhibition, (b) activity of other intestinal membrane transporters.

Keywords: MRP3 drug transporter, regional drug absoprtion, Ussing chamber

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Disruption of the LIN28/Pre-let-7 Axis Through Short Oligonucleotides

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Introduction: LIN28 is an RNA binding protein expressed in ESCs, with multiple roles in development and disease. It is involved in many biological processes, including development, reprogramming, pluripotency, metabolism, tissue regeneration, and tumorigenesis. Humans express two isoforms of LIN28, LIN28A and LIN28B, which bind to the let-7 primary and precursor microRNAs through bipartite recognition, close to the Dicer cleavage site, impeding let-7 biogenesis [1]. It has been shown that in cancer, the tumor suppressor function of let-7 is abrogated by overexpression of LIN28.
Aims: High concentration of LIN28 associates to progress of human malignancies, indicating LIN28 is acting as an oncogene. The NMR structure shows that the two zinc knuckle domains of LIN28 are essential to establish a selective binding with pre-let-7 miRNAs [2]. The structure reveals that each zinc knuckle recognizes an AG dinucleotide separated by a single nucleotide spacer. The proposed project aims to interfere with LIN28, a possible therapeutic oncogene target, through the use of a drug with multiple levels of complexity.
Methods: The project investigates in specific the design of a new drug lead based on a modified 7mer RNA sequence binding to LIN28. The oligonucleotide carries a specific moiety able to recruit a specific proteasome system to achieve targeted degradation of LIN28A [3]. We are currently testing the oligonucleotides constructs in different cancer lines and analyzing the ternary complex LIN28A:Oligo:E3 through biophysical techniques.
Results: We designed a series of short modified oligonucleotides (7-11mers) able to target the binding site of LIN28 with the aim to inhibit the binding of the let-7 precursors through degradation of LIN28A.
Conclusions: Most antisense and RNAi technologies available today aim to correct cancers and genetic diseases at the mRNA level. So far, there is no oligonucleotide tool that could interfere at protein level directly. The nature of Oligo PROTAC makes the new class of oligo a potent drug with action on protein level and represents an innovative oligonucleotides design, with great potential for pharmaceutical development.

Keywords: LIN28A, oligonucleotide, let-7, degradation

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Identification and Pre-Clinical Validation of Optimal Drug Combinations for the Treatment of Metastatic Colorectal Carcinoma Patients

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Introduction: Patients with colorectal carcinoma (CRC) are commonly diagnosed at an advanced stage when the tumor is inoperable and more aggressive. Resection of the primary tumor is followed by adjuvant chemotherapy combinations. However, the 5-year survival rate is only 14% for patients with late stage metastatic disease underlining the need for improved treatment options. In recent years, tyrosine kinase inhibitors (TKIs) have been established as effective targeted therapies. As most curative anti-cancer treatments are combinations of compounds, optimizing drug combinations using TKIs could result in improved treatment options for metastatic CRC (mCRC) patients.
Aim: To develop optimal drug combinations (ODCs) for the treatment of mCRC patients.
Methods: Drug combination optimization using the streamlined-Feedback System Control (s-FSC) method was performed on a panel of CRC cells varying in genetic background, stage and morphology reflecting intra-patient heterogeneity. To optimize clinically relevant drug combinations, screening was simultaneously performed on healthy colon epithelial cells to create opportunities for a therapeutic window. Identified ODCs were validated in complex 3D co-culture models. The efficacy of these ODCs were further investigated in pre-clinical models including *in vivo* and in freshly isolated patient-derived mCRC cells.
Results: Synergistic ODCs were identified for 6 CRC cell lines with varying efficacy on cell viability. The ODCs retained their efficacy in more complex 3D co-cultures. The ODC identified for DLD1 cells was most effective in all CRC cell lines and induced cell cycle arrest and apoptosis. Translation of the ODC to murine tumor model resulted in effective, approx. 80%, tumor growth inhibition. Moreover, the ODCs effective inhibited viability of freshly isolated patient-derived mCRC cells.
Conclusion: Taken together our approach reveals the potential of personalized drug combinations for the treatment of mCRC patients.

Keywords: metastatic colorectal carcinoma, streamlined-Feedback System Control, personalized medicine, drug combinations, therapeutic window

Impact of Intracellular Concentrations on Metabolic Drug-Drug Interaction Studies

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Introduction: Accurate prediction of drug-drug interactions (DDI) is a challenging task in drug discovery and development. It requires determination of enzyme inhibition *in vitro* which is highly system-dependent for many compounds.
Aims: The aim of this study was to investigate whether the determination of intracellular unbound concentrations in primary human hepatocytes can be used to bridge discrepancies between results obtained using human liver microsomes and hepatocytes. Specifically, we investigated if Kp_{uu} could reconcile differences in CYP enzyme inhibition values (K_i or IC_{50}).
Methods: Firstly, our methodology for determination of Kp_{uu} [1] was optimized for human hepatocytes, using 4 well-studied reference compounds. Secondly, the methodology was applied to a series of structurally related CYP2C9 inhibitors from a Roche discovery project. Lastly, the Kp_{uu} values of 3 commonly used CYP3A4 inhibitors - ketoconazole, itraconazole, and posaconazole - were determined and compared to compound-specific hepatic enrichment factors obtained from physiologically based modeling of clinical DDI studies with these 3 compounds.
Results: Kp_{uu} obtained in suspended human hepatocytes gave good predictions of system-dependent differences *in vitro*. The Kp_{uu} was also in fair agreement with the compound-specific hepatic enrichment factors in DDI models [2].
Conclusions: Kp_{uu} can be used to reconcile K_i or IC_{50} values derived from different *in vitro* systems and can improve estimations of enrichment factors in physiologically based pharmacokinetic modeling.

Keywords: drug-drug interaction, intracellular bioavailability, physiologically based pharmacokinetic modeling, scaling factor, unbound drug concentrations

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Antidiabetic Medication, Level of Glycaemic Control, and Risk of Fracture in Patients with Type 2 Diabetes Mellitus: A Nested Case-Control Study

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Introduction: Patients with diabetes mellitus type 2 (T2DM) have an increased risk of low-trauma fractures. However, the effect of antidiabetic medication in association with glycaemic control on the risk of fracture is not well understood.
Aims: To evaluate the association between use of antidiabetic medication, level of glycaemic control, and risk of low-trauma fractures in patients with incident T2DM.
Methods: We conducted an observational nested case-control analysis with patients registered within the UK-based Clinical Practice Research Datalink who had an incident diagnosis of T2DM from 1995-2017. Cases were patients with a low-trauma fracture after the T2DM diagnosis. We matched 4 controls to each case by age, sex, general practice, fracture date, and T2DM duration. Exposure of interest was glycaemic control and any type of diabetes treatment (with or without antidiabetics). We defined exposure categories of antidiabetic treatment according to the NICE guidelines. We assessed glycaemic control as the average HbA1c level during the last 3 years before the fracture. We conducted conditional logistic regression analyses, and adjusted for covariates including body mass index, smoking, and previous fractures.
Results: The study population consisted of 8809 cases and 35219 controls. Patients without DM medication had the lowest mean Hb1Ac levels, while patients treated with the first choice of treatment (metformin), with the first intensification combination therapy (metformin + either DPP4-inhibitor, glitazone, or a sulfonylurea), or patients receiving the drugs recommended as second intensification (triple therapy) had increasingly higher mean HbA1c levels. In patients exposed to metformin (ever-users), very extreme levels of HbA1c (i.e., levels ≤ 6.5% and levels > 9%) were associated with a statistically significantly increased risk of fractures (aOR 1.13, CI 95% 1.03-1.24 and 1.16, CI 95% 1.05-1.28, respectively) compared with patients with HbA1c levels between 6.5-7%. However, for patients receiving any intensification treatment the level of glycaemic control did not impact the risk of fracture when compared with untreated patients.
Conclusions: We observed an association between the use of diabetic medication, HbA1c levels, and the risk of fracture depending on the treatment regimen. While patients receiving a metformin monotherapy and good glycaemic control had a lower risk of fracture, we did not observe an association of glycemic control and the risk of fractures in patients receiving intensified treatment.

Keywords: diabetes, fractures, case-control study, anti-diabetic drugs, epidemiology

Population Pharmacokinetic Modelling for Gentamicin in Pediatric Patients - Towards the Swiss Pharmacokinetics Clinical Data Warehouse *SwissPK^{cdw}*

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Introduction: We are setting up the Swiss Pharmacokinetics Clinical Data Warehouse *SwissPK^{cdw}* for optimizing pediatric dosing regimens based on pharmacokinetic modelling with available clinical data. For a first proof of concept, we addressed the dosing regimen of the aminoglycoside antibiotic gentamicin which is widely used in children. Monitoring of gentamicin serum trough level (C_{min}) is standard practice to prevent toxicity by drug accumulation [1]. C_{min} < 2 mg/L are recommended. Peak serum concentration (C_{max}) is not routinely measured although C_{max} between 10 and 12 mg/L have been recommended balancing efficacy and toxicity [2,3].
Aims: We aimed to evaluate the current dosing regimen for gentamicin in children by population pharmacokinetic (PK) modelling.
Methods: All patients receiving intravenous gentamicin at the University Children's Hospital Zurich between October 2017 and January 2019 were eligible for this study. Children with cystic fibrosis and renal replacement procedures were excluded. Gentamicin was administered once daily at 5 mg/kg in children under 7 days of age and 7.5 mg/kg in older children. Routine C_{min} were measured in all patients before administration of the second or third dose. Additional gentamicin serum levels were measured 30 min (C_{30}) and 4 h after the second dose in patients giving written informed consent. Data were analyzed by non-linear mixed-effects modeling with R.
Results: 165 patients (median age 34 days; IQR 15 to 56 days) were included in the study. A total number of 103 C_{30} and 166 C_{min} measurements were available, respectively. C_{30} (mean 19.7 mg/L, SD +/- 6.1 mg/L) was > 12 mg/L in 94/103 (91%) and C_{min} > 2 mg/mL in 3/166 (1.8%) patients. Non-linear mixed effects modeling was applied to analyse the data further, including body weight, height, age and clinical parameters as potential covariates. Several models successfully predicted most C_{30} > 12 mg/L but performed poorly at the through levels.
Conclusions: The current gentamicin dosing regimen rarely leads to accumulation but most C_{max} are above optimal range. The latter was successfully modelled. Although no evidence for a C_{max} upper limit exists, toxicity has been associated with high drug exposure [3]. This calls for an adjustment of our dosing regimen using our PK model based on body height or body weight in order to lower exposure. Our study highlights the potential of *SwissPK^{cdw}* for optimizing pediatric dosing regimens.

Keywords: gentamicin, pediatric dosing regimens, population pharmacokinetic modeling, Software *R*, *SwissPK^{cdw}*

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