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Schweizerische Zeitschrift für die pharmazeutische Industrie

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Rivista svizzera per l'industria farmaceutica

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University of Bern
20 August 2014
Swiss Academy of Pharmaceutical Sciences (SAPhS)

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SWISS PHARMA 10/14

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CONTENTS

7TH SWISS PHARMA SCIENCE DAY 2014 CONFERENCE REPORT

7TH SWISS PHARMA SCIENCE DAY 2014 PICTURES AND COMMENTS

7TH SWISS PHARMA SCIENCE DAY 2014 POSTER ABSTRACTS

COVER

Amalgam

Amalgam – der bestuntersuchte Werkstoff der restorativen Zahnmedizin
– Prof. Dr. med. dent. K. H. Rateitschak,
Zahnärztliches Institut der Universität Basel

Die unbegründete Angst vor Amalgam
– Prof. Dr. med. dent. J. Wirz,
Zahnärztliches Institut der Universität Basel

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7th SWISS PHARMA SCIENCE DAY 2014

Prof. Dr. Rudolf Brenneisen, University of Bern, Swiss Academy of Pharmaceutical Sciences (SAPhS)
Dr. Benoîte Kaeser, Basel, Swiss Academy of Pharmaceutical Sciences (SAPhS)

At its inception seven years ago, the Swiss Pharma Science Day was intended as a gathering of Swiss pharmaceutical scientists working in academia and industry, with a special focus on the support of young scientists. This idea has been met with enthusiasm by pharmaceutical scientists and the support by the pharmaceutical industry and regulatory authorities. The organizers were thus highly motivated to make this year's event again a success for the participants, and pharmaceutical sciences in Switzerland as such. Here is our report on the 7th SWISS PHARMA SCIENCE DAY of August 20, 2014 held again at the Pathology Langhans Auditorium of the University of Bern.

Dr. Benoîte Kaeser, secretary and SAPhS board member, Christiane Borchard, and Prof. Georg Imanidis, SAPhS board member, ready to welcome participants.

Dr. Christine Moll together with Proff. Bruno Gander and Georg Imanidis, SAPhS board members, preparing the registration desk.

7th SPhSD 2014

Addresses of welcome

– Prof. Dr. Gerrit Borchard, President SAPhS
– Prof. Dr. Hugues Abriel, Director Dep. Clinical Research (DCR), University of Bern
– Prof. Dr. Rudolf Brenneisen, General Secretary SAPhS

The seventh edition of the Swiss Pharma Science Day (SPhSD) took place on August 20, 2014, and was again a very successful event. The conference, organized by the Swiss Academy of Pharmaceutical Sciences (SAPhS) by Proff. R. Brenneisen, University of Bern, and G. Borchard, University of Geneva, was held again at the University of Bern. The program of plenary lectures, interrupted by the poster session, attracted approximately 180 scientists mainly from

Swiss universities and pharmaceutical industry. The conference was opened by Prof. Gerrit Borchard, President SAPhS, and Prof. Hugues Abriel, Director DCR, who welcomed the participants at the premises of the University of Bern.

Audience, waiting for opening ceremony.

Prof. Gerrit Borchard, President SAPhS.

Prof. Hugues Abriel, Director Dep. Clinical Research, University of Bern.

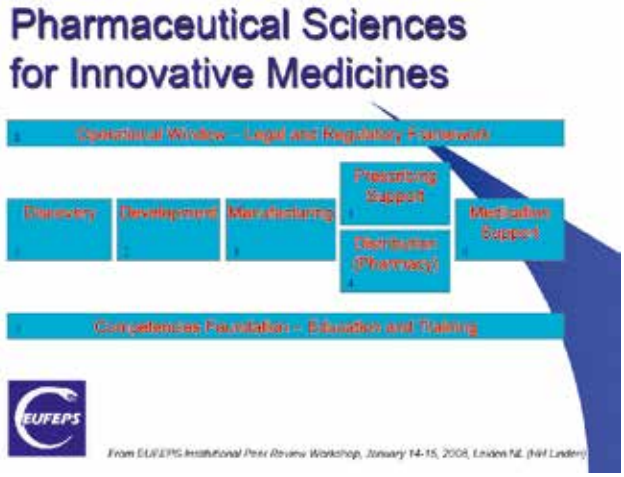
The morning session was chaired by Prof. Rudolf Brenneisen, organizer of the SPhSD. He titled the topics of the forthcoming sessions as "The Brain Day" with lectures on the experience with CNS-active drugs (melatonin, psychoactive substances), an introduction to the ambitious "Human Brain Project", and a lecture on the development of future medicines. The program was rounded off with a lecture on omega-3-fats as an example for neutraceuticals, and a presentation on the national and international activities of the Spiez Laboratory dealing with Nuclear, Biological, and Chemical Protection (NBC), which could be described as "Science for Peace".
The first and keynote lecture "Reflections on the Future of Pharmaceutical Sciences" was given by Prof. em. Dr. Daan Crommelin, Utrecht University. Based on his tremendous experience as professor for Pharmaceutics and Pharmaceutical Chemistry and as contributor to the development of several innovative product formulations, he presented examples of successful achievements of safe and effective drugs in the past 50 years, such as oral contraceptives, levodopa, OROS-systems, and pegylation of proteins. For the future, he encouraged scientists in industry and academia to enhance collaboration and joint venture activities allowing also new and unconventional methods in the field of pharmaceutical research and development.

Lecture 1: Keynote Speech

Prof. em. Dr. Daan Crommelin, Utrecht University: "Reflections on the Future of the Pharmaceutical Sciences"

Prof. Daan Crommelin, Utrecht University.

To start his "reflections", a definition of the pharmaceutical sciences was given by a diagram that was presented by the European Federation of Pharmaceutical Sciences, EUFEPS, in 2008.

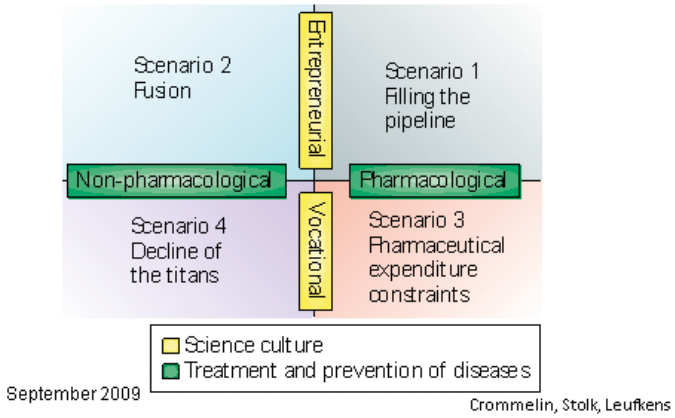


Knowledge transfer between the different blocks is not unidirectional, but in both directions. The top and bottom bars are an integral part of the diagram. The legal and regulatory framework is essential to understand the way medicines are developed, dispensed and used. This has implications for the "competence foundation": education and training. One should realize that the pharmaceutical sciences are based on many different scientific pillars and have as a main task to amalgamate those to improve pharmacotherapy for the patient. That laudable goal ("amalgamating") is not always understood and appreciated by policy makers and academic funding authorities. Learned societies at the national and international level, such as EUFEPS, should keep on drawing attention to this unique element of the pharmaceutical sciences.

Recently, an article was published with the title: "Impact of the Pharmaceutical Sciences on Health Care: A Reflection over the Past 50 years" (Rowland et al., J. Pharm. Sci. 2013). In that document the impressive progress made in the last 50 years has been documented. But the number of hurdles and challenges is growing. For example, the "low hanging fruit" has been picked and society is becoming more and more risk averse (cf. "precautionary principle"). Other scholars are very explicit in their views about the future and challenges that we face, such as James Le Fanu in his "Rise and Fall of Modern Medicine", 2012.

The question is now: How will the pharmaceutical world look like in the year 2020 or 2030? One can follow the old Greek way and consult oracles or astrologists. A more recent approach is to perform a scenario analysis as is done regularly in industry and large organizations. These analyses are not forecasts but learning devices to open "mental maps". And that was the endeavor the Board of Pharmaceutical Sciences (BPS) of the International Pharmaceutical Federation (FIP) embarked on. 30+ expert with different background and from different parts of the world identified drivers influencing the future of the pharmaceutical sciences and the group worked out four scenarios for the pharmaceutical sciences. The outcome of these four different scenarios, including narratives for different subgroups, was published, a.o. in Nature Rev. Drug Discovery 2010, cf. scheme 1, and by Shah et al., Eur. J. Pharm. Sci. 2009.

The 2 x 2 scenario matrix is the basis for the 4 scenario narratives



Scheme 1

The main drivers for future developments were identified by participants and are listed below (not in order of priority):

1. Molecular biology, biomarkers, genomics
2. Information and other new (material) technologies
3. Rethinking the pharma business model
4. Funding and investing models, risk sharing
5. Regulatory constraints and opportunities
6. Health care environment, payers in the hot seat
7. Therapeutic gaps, unequal access
8. Patient/public advocacy, zero risk culture, ethics
9. Demographics, longevity, chronic diseases
10. Globalization, emerging markets (BRIC countries)
11. Financial system

In this lecture attention was paid to the expected impact of a selected number of these drivers. The continuing fast advances in molecular biology/analytical techniques which open the way for detailed genotypic/phenotypic analysis of material of patients and new prescribing paradigms such as personalized medicine or, maybe more realistically, "stratification" of therapeutic interventions in target patient groups. Molecular biology will also improve taxonomy of human diseases and that will again impact the prescribing routines. Another trend that has already started is the "blurring of border lines" between presently (basically) separated fields, e.g. between pharmacotherapy, imaging and other diagnostic technologies. And the continuously increasing computing power will lead to advanced modeling and systems approaches. "Big data" handling strategies also help to further evaluate large data sets on patient medicine utilization (epidemiology).

The coming years, the business model behind the development, including the approval process, of new medicines, will continue to move away from the "closed innovation model" to "the open innovation model". "Big pharma" is withdrawing from performing basic research in house and focuses on (clinical) development and manufacturing, leaving the real breakthrough innovations to SMEs (small and medium sized companies) and the academic world. It is essential that the proper infrastructure is developed for the flow of information between the players/stakeholders (including regulatory bodies) in the open innovation model. The formation of public private partnerships (PPP) such as the Dutch Top Institute Pharma (at the national level) and the Innovative Medicines Initiative (IMI I and II) at the European level is a logical (and critical) step to make the "open innovation model" successful.

The following talk "Toxicokinetics of Older and Novel Psychoactive Substances", given by Prof. Hans Maurer, Saarland University, Homburg, impressed by showing the possibilities of laboratory and analytical methods in elucidating the structure of known and unknown compounds, used as recreational drugs, and their metabolism. In addition the application of genotyping and phenotyping methods helps understanding drug metabolism in the individual patient and can be used to understand or prevent over- or underdosing when administered alone or in combination with other drugs (drug interactions).

Lecture 2: Toxicology

Prof. Dr. Hans Maurer, University of Saarland, Homburg/Saar: "Toxicokinetics of Older and Novel Psychoactive Substances"

Prof. Hans Maurer, University of Saarland.

Individual variations in the pharmacological responses to the same drug dose may be caused by a variety of factors such as body mass, age, sex, kidney and liver function, drug-drug (food-drug) interactions, or genetic variability. Detailed knowledge of the metabolism and membrane transport of drugs allows predicting possible interactions with other xenobiotics because of e.g. inhibition or induction of individual metabolic isoenzymes by poisons, drugs (of abuse), alcohol, or ingredients of tobacco or food [1-4]. Hence, understanding pharmacokinetic and pharmacogenomic variations is a prerequisite for evidence-based case interpretation, for toxicological risk assessment, for developing toxicological analysis procedures, and for understanding pitfalls in drug testing. In the presentation, the major metabolic pathways and the involved isoenzymes in humans will be summarized for the major drugs of abuse. It will also provide an overview on the implications of the presented data for possible interactions of drugs of abuse with other xenobiotics, i.e. inhibition or induction of individual polymorphic and non-polymorphic drug metabolizing and/or transport isoenzymes.

References:

[1] Maurer HH. Toxicokinetics – Variations due to Genetics or Interactions: Basics and Examples. In Current Contributions to Forensic and Clinical Toxicology, Pragst, F., Aderjan, R., Eds.; GTFCH: Bad Vilbel (Germany), 2008; pp 153-155.
[2] Meyer MR, Maurer HH. Metabolism of designer drugs of abuse: An updated review [review]. Curr Drug Metab 2010; 11: 468-482.
[3] Meyer MR, Maurer HH. Absorption, distribution, metabolism and excretion pharmacogenomics of drugs of abuse [Review]. Pharmacogenomics 2011; 12: 215-233.
[4] Meyer MR, Orschiedt T, Maurer HH. Michaelis-Menten kinetic analysis of drugs of abuse to estimate their affinity to human P-glycoprotein. Toxicol Lett 2012; 217: 137-142.

The presentation of Dr. Marc Cadisch, Director of the Federal Spiez Laboratory, was on "Swiss Science and Technology for NBC Protection". He demonstrated the importance of interdisciplinary collaboration of scientists (including biologists, physicists, and chemists) in the field of nuclear, biological and chemical (NBC) protection. The institution is recognized worldwide and was also charged by the UN/OPCW (United Nations / Organisation for the Prohibition of Chemical Weapons) with the investigation on the use of chemical weapons in the Syria civil war in 2013. The 2013 Nobel Peace Prize was awarded to the OPCW, therefore also partly to the Spiez Laboratory!

Lecture 3: Organic Chemistry

Dr. Marc Cadisch, Spiez Laboratory, Spiez: "Spiez Laboratory – Swiss Science and Techology for NBC Protection"

Dr. Marc Cadisch, Spiez Laboratory.

Established in 1925, the Swiss Federal Institute for NBC-Protection deals with the risks and dangers of nuclear, biological and chemical weapons, constantly developing and safeguarding the necessary scientific and technological knowledge for comprehensive protective measures. However, the strategic focus has expanded in the past years: Whereas in the early days the emphasis was on protective measures for armed forces, todays' activities also support international arms-control and non-proliferation initiatives, such as the analytical work for the UN/OPCW investigation in Syria in 2013. The "Spiez Laboratory" was one of the laboratories providing the scientifically irrefutable proof of the use of chemical weapons in the Syrian civil war, employing parallel analysis with nuclear magnetic resonance spectrometry, gas chromatography with specific detection systems, such as mass spectrometry or atomic emission detector, as well as liquid chromatography coupled to mass spectrometry. The UN/OPCW mission in Syria has demonstrated the importance of multilateral disarmament organizations. However, without solid factual knowledge, progress in arms-control negotiations is difficult. As a designated laboratory of the OPCW, the IAEA and the ICRC, the "Spiez Laboratory"occupies an important interface between science and arms-control policy. One of its central tasks is keeping track of developments in science, anticipating their impact on civil protection and initiating the necessary measures. In this context, the "Spiez Laboratory"established a series of international conferences under the title "Spiez Convergence", in order to address the partly overlapping developments in biology and chemistry and to indicate possible consequences for the implementation of the chemical and biological weapons conventions.

Poster Session

After the lunch break with an excellent Chinese food buffet, the participants were invited to the poster session in order to exchange scientific knowledge with young academics and to socialize. The abstracts of 71 posters can be found at the end of this article. As in previous years, 6 authors were evaluated by the reviewing board to receive awards for outstanding poster presentations. These awards were sponsored by the AKB Foundation, GSIA Foundation, Pharmaceutical Society of Zurich, TTC Glatt Group, Zeller Söhne AG, and Vifor Pharma.

Outdoor lunch break.

Prof. Jürgen Drewe (Zeller Söhne AG) and Beat Meier (ZHAW).

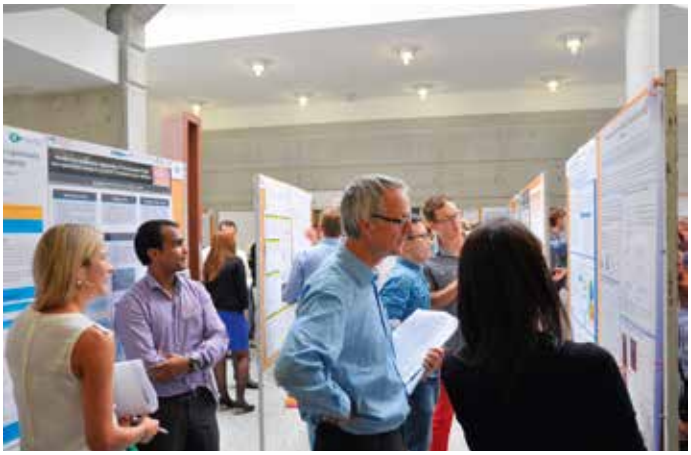
Prof. Ueli Honegger, Fellow SAPhS, and Michele Bordoni, President AKB.

Prof. Stefan Mühlebach, Vice-president SAPhS, and Dr. Marc Cadisch, head Spiez Laboratory.

PD Olivier Potterat, University of Basel, in discussion with participants.

Prof. Georg Imanidis, FHNW, SAPhS board member.

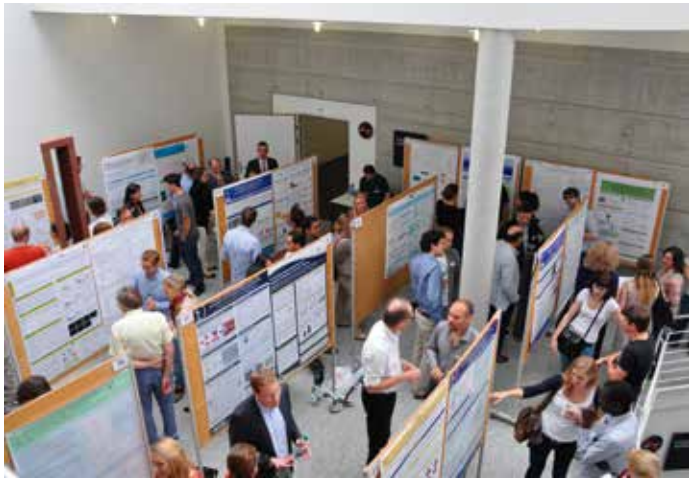
Prof. Kurt Hersberger, University of Basel and SAPhS Fellow 2014, together with Dr. Christian Jaeggi, Cincap Basel.



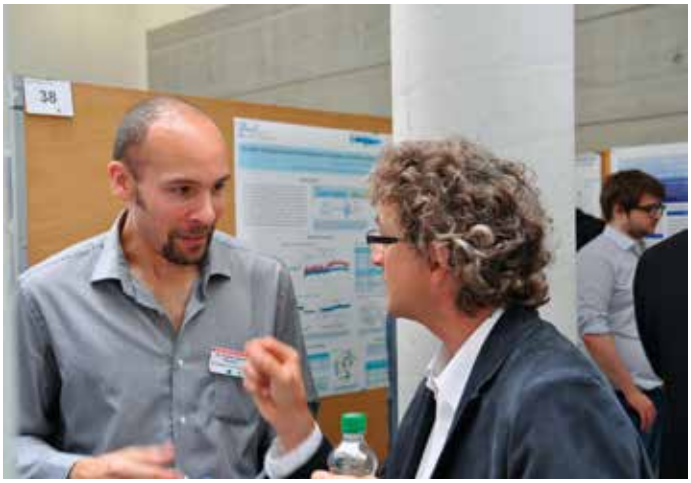
Prof. Bruno Gander, member of SAPhS board and poster reviewer.

Philippe Tschopp, SAPhS board member and SPhSD photographer.

Prof. Muriel Cuendet, University of Geneva, poster reviewer.

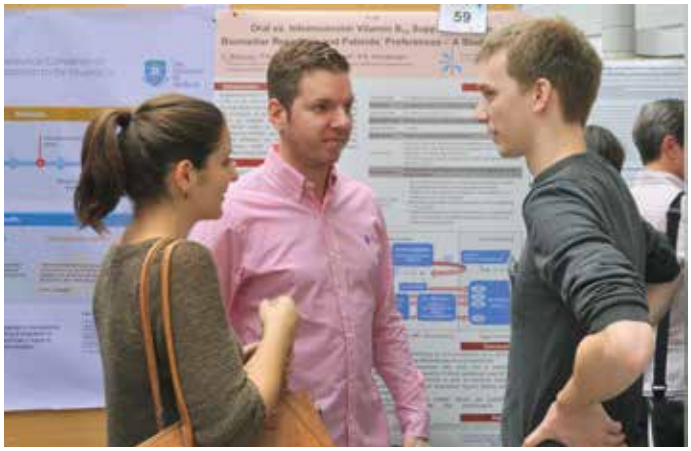


Poster session starting.



Dr. Romain-Daniel Gosselin, Biotelligences LLC Lausanne, in discussion with Prof. Nicolas Schaad, University of Geneva.

Jaqueline Bezençon, University of Basel, poster presenter.



Young scientists discussing poster.

Colleagues from ETHZ and University of Geneva.

Prof. Gerrit Borchard chaired the afternoon session. First, Prof. Philip Calder, University of Southampton, gave a lecture on "Omega-3 Fats as Nutraceuticals: From Science to Practical Use". Most of his research over the past 25 years was dedicated to the metabolism and functioning of fatty acids. In humans the beneficial effects of omega-3 fatty acids (O3FA) are, among others, on lowering blood pressure and plasma triglycerides, lowering the risk of myocardial infarction, improving cardiovascular function, and reducing inflammatory action, such as in rheumatoid arthritis, inflammatory bowel disease, asthma, COPD, and psoriasis. He recommended supplementation with O3FA (e.g. 900 mg daily). The highest contents of O3FA in food were measured in tuna, salmon, and sardines (1-3%).

Lecture 4: Nutrition Sciences

Prof. Dr. Philip Calder, University of Southampton: "Omega-3-Fats as Nutraceuticals: From Science to Practical Use"

Prof. Philip Calder, University of Southampton.

Omega-3 fatty acids (O3FA) are a family of polyunsaturated fatty acids that contribute to human health and well-being. Functionally the most important O3FA appear to be eicosapentaenoic acid (EPA) and docosahexaenoioc acid (DHA) found in oily fish and in supplements, but roles for docosapentaenoic acid (DPAn-3) are emerging. Intakes of EPA and DHA are typically low and much below recommended intakes. Increased intakes are reflected in greater incorporation into blood lipid, cell and tissue pools. Increased content of EPA and DHA can modify the structure of cell membranes and also the function of membrane proteins involved as receptors, signaling proteins, transporters and enzymes. EPA and DHA also modify the production of lipid mediators and through effects on cell signaling can alter patterns of gene expression. Through these actions EPA and DHA act to alter cellular responsiveness in a manner that seems to result in more optimal conditions for growth, development and maintenance of health. The effects of O3FA are evident right through the life course meaning that there is a need for all sectors of the population to increase the intake of these important nutrients. EPA and DHA have a wide range of physiological roles which are linked to certain health or clinical benefits. A number of risk factors for cardio-vascular disease are modified in a beneficial way by increased intake of EPA and DHA: these include blood pressure, platelet reactivity and thrombosis, plasma triglyceride concentrations, vascular function, cardiac arrhythmias, heart rate variability, and inflammation. As a result of these effects, increased EPA and DHA intake is associated with a reduced risk of cardiovascular morbidity and mortality. Thus, there is a key role for these fatty acids in prevention and slowing progression of cardiovascular disease. Furthermore, some supplementation studies with EPA and DHA have demonstrated reduced mortality in risk patients, such as post-myocardial infarction, indicating a therapeutic role. A number of other, non-cardiovascular, actions of EPA and DHA have also been docu-

mented, suggesting that increased intake of these fatty acids could be of benefit in reducing the risk of (i.e. protecting from) or treating many conditions. For example, they have been used successfully in rheumatoid arthritis and, in some studies, in inflammatory bowel diseases, and may be useful in other inflammatory conditions like asthma, chronic obstructive pulmonary disease, and psoriasis. EPA and DHA may also have a role as part of cancer therapy; some recent studies show that they improve the effectiveness of some chemotherapeutic agents. DHA has an important structural role in the eye and brain, and its supply early in life when these tissues are developing is known to be of vital importance in terms of optimizing visual and neurological development. For this reason it is very important that pregnant and breast feeding women have adequate DHA intake. Recent studies have highlighted the potential for EPA and DHA to contribute to enhanced mental development and improved childhood learning and behaviour and to reduce the burden of psychiatric illnesses in adults, although these areas of possible action require more robust scientific support. There may also be a role for EPA and DHA in preventing neurodegenerative disease of ageing. The effects of EPA and DHA on health outcomes are likely to be dose-dependent, but clear dose response data have not been identified in most cases. Also in many cases it is not clear whether both EPA and DHA have the same effect or potency and therefore which one will be the most important for a particular indication.

Prof. Nicolas Schaad, Pharmacie Interhospitalière de la Côte, Morges, and University of Geneva, was then talking about "Melatonin – the Queen of the Night" and its effects on the circadian rhythm. To date melatonin and several melatonin analogues are registered in Switzerland as safe sleep inducing agents for the treatment in elderly patients and children. In addition, treatment with melatonin and analogues show beneficial effects on restoration of the circadian rhythm in blind individuals.

Lecture 5: Pharmacology

Prof. Dr. Nicolas Schaad, Pharmacie Interhospitalière de la Côte, Morges, and University of Geneva: "Melatonin – The Queen of the Night"

Prof. Nicolas Schaad, Pharmacie Interhospitalière de la Côte and University of Geneva.

The mammalian pineal gland secretes melatonin during the dark phase of the circadian rhythm which is generated by the main biological clock located within the suprachiasmatic nuclei (SCN) of the hypothalamus. In men, the daily amount of melatonin secreted by the pineal gland is lower than 100 µg. Melatonin is converted from blood serotonin, but does not share its pharmacology. The actions of melatonin are diverse. It is known for more than a century that a pineal gland extract possesses the ability to lighten frog skin. A dermatologist who isolated melatonin in 1950 from bovine pineal glands shortly noticed that this hormone had no effect on human skin, but had a sedative effect when absorbed orally. Melatonin has at least 2 subtypes of high affinity receptors, and a high density is found within the SCN. It seems therefore that melatonin could play an important role in the regulation of circadian rhythms. Melatonin has been tested in several human pathologies, but very few results have been confirmed in large, high-quality clinical trials. This hormone is effective for the treatment of the jet lag, which was originally described by Post and Gatty after the first around the globe flight in 1931. After a transmeridian flight, our circadian rhythms are heavily perturbated, and melatonin helps to resynchronize our biological clock. Melatonin has also an effect on sleep. Most of the studies conducted in men show that melatonin reduces sleep latency and increases sleep efficiency. The mechanism by which melatonin promotes sleep is not totally elucidated, but could be secondary to a hypothermic effect or by promoting the opening of the "gate of sleep". Several groups of patients (blind people, children, elderly) could benefit from a melatonin treatment, whose safety profile is good. This hormone is registered by Swissmedic for a couple of years and several melatonin analogues are now available on the market.

The last lecture was given by Prof. Richard Frackowiak, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, on "The Human Brain Project: Implications for Pharma with Reference to Neurodegeneration". This project consists of interdisciplinary efforts of teams working in brain imaging technologies, biological, genetic and molecular data mining combined with information technologies in order to define (through simulation and modelling) the processes and causes which lead to the development of psychiatric and neurological diseases. Scientists from 22 countries across Europe, the USA, and Asia contribute to this ambitious target. The project was launched in October 2013, and the duration is planned for 10 years with regular review cycles. The outputs of the project should, of course, also provide tools and information for the prevention and treatment of psychiatric and neurological diseases.

Lecture 6: Neurosciences

Prof. Dr. Richard Frackowiak, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne: "The Human Brain Project: Implications for Pharma with Reference to Neurodegeneration"

Prof. Richard Frackowiak, CHUV.

We now know that a single gene mutation may present with multiple phenotypes, and vice versa, that a range of genetic abnormalities may cause a single phenotype. These observations lead to the conclusion that a deeper understanding is needed of the way changes at one spatial or temporal level of organisation (e.g., genetic, proteomic or metabolic) integrate and translate into others, eventually resulting in behaviour and cognition. The traditional approach to determining disease nosology - eliciting symptoms and signs, creating clusters of like individuals and defining diseases primarily on those criteria has not generated fundamental breakthroughs in under-standing sequences of pathophysiology mechanisms that lead to the repertoire of psychiatric and neurological diseases.
It is time to radically overhaul our epistemological approach to such problems. We now know a great deal about brain structure and function. From genes, through functional protein expression, to cerebral networks and functionally specialised areas defined via physiological cell recording, microanatomy and imaging we have accumulated a mass of knowledge about the brain that so far defies easy interpretation. Advances in information technologies, from supercomputers to distributed and interactive databases, now provide a way to federate very large and diverse datasets and to integrate them via predictive data-led analyses.
Human functional and structural brain imaging with MRI continues to revolutionise tissue characterisation from development, through ageing and as a function of disease. Multi-modal and multi-sequence imaging approaches that measure different aspects of tissue integrity are leading to a rich mesoscopic-level characterisation of brain tissue properties. Novel image classification techniques that capitalise on advanced machine learning techniques and powerful computers are opening the road to individual brain analysis. Data-mining methods, often developed in other data-rich domains of science, especially particle and nuclear physics, are making it possible to identify causes of disease or its expression from patterns derived by exhaustive analysis of combinations of genetic, molecular, clinical, behavioural and other biological data. Imaging is generating data that links molecular and cellular levels of organisation to the systems that subtend, action, sensation, cognition and emotion. An additional feature will be that therapeutic target identification will be facilitated and study cohorts will be much cleaner and well-defined, resulting in smaller, more powerful drug trials. These ideas will be illustrated with reference to the human dementias.

Prof. Richard Frackowiak receiving a sweet present from the SAPhS President.

Recognitions and Awards

Fellows 2014

The SAPhS consists of scientists who are distinguished by their outstanding research and professional contributions in the field of Pharmaceutical Sciences in Switzerland. The following four distinguished scientists were awarded "Fellows of the SAPhS:

Dr. Colette Andrée, University of Basel, initiator and managing director of the Migraine Working Group, "For her contributions in patient-oriented pharmacy in multidisciplinary projects in the health care system to develop the pharmaceutical sciences and education and for her active role in SSPhS and SAPhS".

Prof. em. Dr. Hans Leuenberger, Ifiip GmbH, Pfeffingen, former head of Pharmaceutical Technology, University of Basel, "For his advancing Pharmaceutical Sciences in Switzerland, his contributions in the areas of Physical Pharmacy, Pharmaceutical Technology and Industrial Pharmacy and his efforts to establish and develop the SSPhS and SAPhS".

Prof. Dr. Beat Meier, University of Applied Sciences, Wädenswil Zurich (ZHAW) "For his achievements in Phytochemistry, Phytopharmacy and Phytotherapy in education, research and industry".

Prof. Dr. Kurt Hersberger, Pharmaceutical Care Group, University of Basel, "For his contributions to the field of Clinical Pharmacy, Pharmaceutical Care and Drug Safety in Switzerland".

SAPhS Fellows 2014 (from left): Prof. Hans Leuenberger, Colette Andrée, Prof. Beat Meier.

Poster award winners

1st Prize, sponsored by the AKB Foundation:
Stefanie Haller, ETHZ-PSI-USZ
Poster P-13: "Evaluation of the Chick Embryo as an *In Vivo* Test System for Radiopharmaceuticals"

2nd Prize, sponsored by the GSIA Foundation:
Stella-Saphira Ehrenberger, University of Geneva
P-63: „Local Delivery of Superparamagnetic Nanoparticles for Application of Local Hyperthermia in Cancer"

3rd Prize, sponsored by the Pharmazeutische Gesellschaft Zürich (PharmGZ):
Martina Roos, ETHZ
P-71: "Development of a FRET-based HTS for the Identification of Lin28/pre-let-7 Interaction Antagonists"

Prize for best poster in Pharmaceutical Technology, sponsored by the TTC Glatt Group:
Jan Kendall de Kruif, University of Basel
P-65: "Prilled Microgels in Lipid Dispersions for Oral Delivery of Biologicals"

Prize for best poster in Pharmaceutical Biology, sponsored by Zeller Söhne AG:
Sylvian Cretton, University of Geneva
P-32: "Antitrypanosomal Activity of Quinoline Alkaloids from the Roots of Waltheria Indica"

Special prize, sponsored by Vifor Pharma:
Romain-Daniel Gosselin, Biotelligences LLC Lausanne
P-33: "Misuse of Statistics in Experimental Pharmacology – An Evaluation of Six Journals"

Poster award winners (from left): Stefanie Haller, ETHZ (1st prize); Jan Kendall de Cruif, University of Basel (technology prize); Stella-Saphira Ehrenberger, University of Geneva (2nd prize); Sylvian Cretton, University of Geneva (pharm. biology prize); Romain-Daniel Gosselin, Biotelligences LLC Lausanne (special prize); Martina Roos, ETHZ (3rd prize).

Thanking and invitation to the 8th Swiss Pharma Science Day on Wednesday August 19, 2015

The organizers would like to thank all speakers for their excellent presentations, and the Faculty of Medicine of the University of Bern as the host of this event. The AKB Foundation (gold sponsor), GSIA Foundation (silver sponsor), Mundipharma Medical Comp. (silver sponsor), CSL Behring AG, Galexis AG, Pharmaceutical Society of Zürich, TTC Glatt Group, Max Zeller Söhne AG, Vifor Pharma, Verlag Dr. Felix Wüst AG, Küsnacht ZH, and pharmaSuisse are acknowledged for their continued financial support.
The organizers are looking forward to welcome young pharmaceutical scientists to the 8th edition of the SPhSD on August 19, 2015, again in Bern and at the same location.
It is a tradition to finish the Swiss Pharma Science Day with an apéro at the beautiful setting of the House of the University of Bern, which allows relaxing from the dense scientific program and socializing.

Vroni Jakob, PharmGZ, and Prof. Beat Meier, ZHAW, enjoying the excellent white wine.

Dr. Jaeggi together with Proff. Drewe, Huwyler and Leuenberger (from left).

Apéro outdoor, view to the Kocher park.

Prof. Rudolf Brenneisen, University of Bern
Prof. Gerrit Borchard, University of Geneva
Organizers SPhSD

SWISS PHARMA SCIENCE DAY 2014

Poster Abstracts

P-1

Pharmaceutical Quality of Eight Generics of Ceftriaxone Preparation for Injection in Eastern Asia

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[2] *F. Hoffmann-La Roche Ltd, Basel, Switzerland*

Introduction: Ceftriaxone is a broad-spectrum bactericidal agent of the third-generation cephalo-sporins listed by the WHO as an essential medicine [1]. By definition, a generic product is expected to demonstrate the same qualitative and quantitative composition in active substances, the same pharmaceutical form and bioequivalence with the innovator brand product to be considered interchangeable with it. For parenteral formulations, therapeutic equivalence is assumed from pharmaceutical equivalence. Clinical and microbiological failures were reported with generic antibiotics [2], as well as bacterial resistance [3].
Aims: To compare the pharmaceutical quality of 8 generic products manufactured in Eastern Asia to the original Rocephin® 1000 mg preparation for injection.
Methods: Original ceftriaxone (Rocephin®, F. Hoffmann-La Roche, Basel, Switzerland) was used as reference material. Generics produced in Taiwan, China, India and Indonesia were purchased from approved commercial supply channels in China and Myanmar. The following tests were performed: crystallinity and color of powder, presence of particles, opalescence and pH of solution, filling content, impurities, residual solvents and heavy metals, sterility and microbiological contamination. Results were compared to the manufacturer's specifications for the originator and the European Pharmacopoeia.
Results: Eight generic products were purchased in January 2013 and analyzed in March-October 2013 within their expiration dates. All 8 generic products had an amorphous appearance in contrast to the crystalline reference, and all failed the specifications in 3 or more tests. Four generic products were not particle-free (2 manufactured in India, 1 in China, 1 in Indonesia), 8 showed impurities as lubricants or solvents, and 2 were not sterile (from India). Residues of heavy metals were detected in all generics, in quantities ranging from 1 ppm (Zn) to 16 ppm (Br).
Conclusions: All tested generic ceftriaxone products manufactured in Eastern Asia failed to meet *in vitro* quality compared to the original branded product. The impurities identified as particles, residues of solvents and metals, as well as microbiological contamination are of clinical concern, as they could impact tolerability and safety in patients in need of an effective parenteral antibiotic.

Keywords: Ceftriaxone, rocephin, pharmaceutical quality, generics, Eastern Asia.

References:
[1] World Health Organization. WHO model list of essential medicines, 18th list; 2013.
[2] Mastoraki E et al. J Infect 2008; 56: 35-9.
[3] Rodriguez CA et al. Antimicrob Agents Chemother 2012; 56: 243-7.

P-2

Reversible and Tunable PEGylation at Arginine Residues

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Introduction: In the last couple of decades, conjugating methoxy poly(ethylene glycol) (mPEG) to proteins (PEGylation) has been exploited to prevent their fast recognition and high overall clearance rate by the immune system. However protein bioactivity can be severely comprised in this process. Therefore, the "tunable" release of native (and fully bioactive) proteins from protein-polymer conjugates (rPEGylation) is attracting increased research interest. New chemistries are required for making this process efficient and convenient. Phenylglyoxal (PGO), an aromatic α-oxo-aldehyde, selectively reacts with arginine residues in a pH-dependent manner. The PGO-arginine link is known to be labile, allowing the reversible PEGylation at arginine, a residue for which rPEGylation has not yet been reported.
Aims: A novel platform for selective and reversible modification of arginine residues with PGO will be developed and evaluated, as shown in Figure 1a.

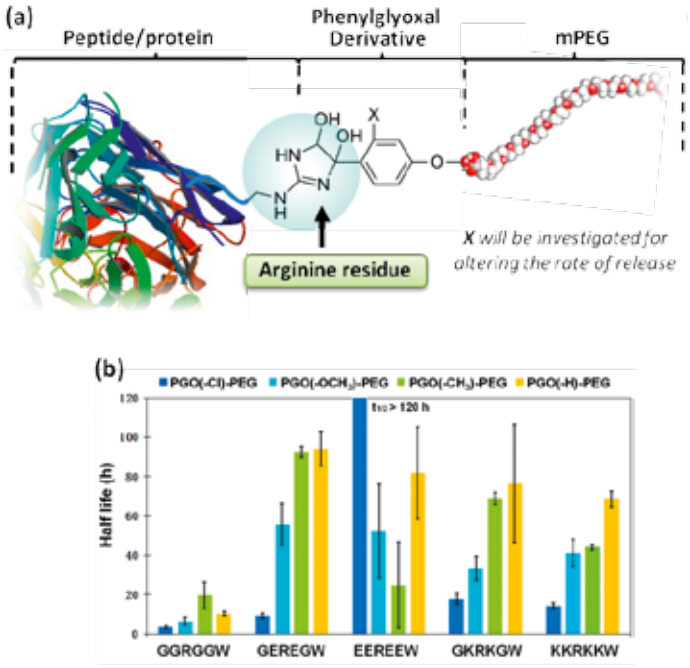

Figure 1 (a) Example of a PGO-PEG-protein system.
(b) Half life of reactions of peptides with different PGO-PEG

Methods: Derivatives of PEG bearing a PGO unit modified with different functional groups (-H, -Cl, $-CH_3$ and $-OCH_3$) [m.wt. ≈ 570 g/mol] were synthesized. The PGO-PEG adducts were characterized by

NMR and MS. The resulting reactive PEGs are expected to react with peptides containing arginine residues. Therefore, several short peptides with variable charges surrounding the central arginine (GGRGGW, GEREGW, EEREEW, GKRKGW and KKRKKW) were synthesized and modified with the PGO-PEG derivatives. Kinetic studies (5 days) were monitored by HPLC-MS and HPLC. Changes in the concentration of the following species were assessed: free peptide, PGO-PEG and peptide-PGO-PEG.
Results: Kinetic studies showed that the decreases in the reactants were always accompanied by the increases in the target products. Modification of the PGO unit resulted in different reaction rates, with -Cl being the fastest and -H being the slowest. Peptide sequence also had an influence on the reaction rate, indicating that local pH and steric hindrance might play a role in the coupling reaction (Figure 1b).
Conclusions: Results obtained thus far indicate that it is possible to manipulate the rate of coupling between PGO-PEG and arginine residues. This work therefore appears promising for selective conjugation at arginine residues with entities, such as polymers or small molecule ligands. Future work will focus on evaluating the influence of the modification to PGO on the rate of release of the native peptide under physiological conditions (rPEGylation).

Keywords: Conjugated peptide, phenylglyoxal, rPEGylation.

References:
[1] Gauthier MA et al. Polym Chem 2011; 2: 1490-1498.
[2] Gauthier MA, Klok H. Biomacromolecules 2011; 12: 482-493.
[3] Fuhrmann G et al. Nat Chem 2013; 5: 582-589

P-3

Snapshot Pharmacokinetic Studies in Mice of Two Bioactive Piperine Derivatives with γ-Aminobutyric Acid Type A ($GABA_A$) Receptor Modulatory Properties

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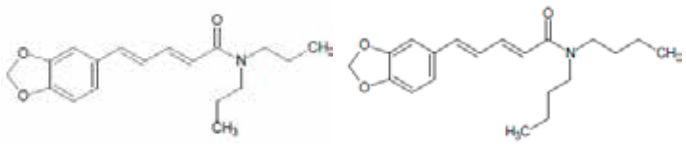
Introduction: Piperine was recently identified as a new scaffold of $GABA_A$ receptor ($GABA_AR$) modulators [1]. A library of 76 piperine analogues was synthesized and screened, and two compounds were selected for assessment of first PK data. Compound **23** [*(2E,4E)*-5-(1,3-benzodioxol-5-yl)]-*N,N*-dipropyl-2,4-pentadienamide] induced the strongest modulation of $GABA_A$, while compound **25** [*(2E,4E)*-5-(1,3-benzodioxol-5-yl)-*N,N*-dibutyl-2,4-pentadienamide] showed the highest potency [2].
Aims: In order to reduce time and costs for compound screening, a high-throughput PK approach omitting bioanalytical method validation [3] was used for a rapid estimation of main pharmaco-kinetic parameters in K3 EDTA C57BL/6N mouse plasma.
Methods: Blood samples from male C57BL/6N mice (6 months) were taken at 15, 30 and 60 min after i.p. application of **23** and **25** (doses 1, 3 and 10 mg/kg b.wt.). After protein precipitation of plasma samples, quantification was performed on a 1290 Infinity UHPLC system coupled with a 6460 triple quadrupole mass spectrometer (Agilent technologies) using multiple reaction monitoring (MRM) in positive ESI mode. Data were analyzed by non-compartmental analysis using PKSolver 2.0.
Results: The estimated plasma concentrations were below the µM concentrations required for significant I_{GABA} potentiation of $GABA_AR$ expressed in *Xenopus* oocytes. The apparent volumes of distribution Vd (between 7.69 and 12.1 L/kg) for both compounds were large, indicating that they were mainly distributed into deeper tissues. Compound **23** showed dose-dependent elimination, whereas compound **25** followed first-order kinetics.



(23) **(25)**

Figure 1 Compound 23 [(2E,4E)-5-(1,3-benzodioxol-5-yl))-N,N-dipropyl-2,4-pentadienamide] and compound 25 [(2E,4E)-5-(1,3-benzodioxol-5-yl)-N,N-dibutyl-2,4-pentadienamide]

Conclusions: Metabolite formation of compounds **23** and **25** is unknown at present, and we cannot exclude that the observed anxiolytic and sedative effects are induced by more active metabolites. Investigation of blood brain barrier permeation and tissue accumulation of both compounds are in progress.

Keywords: Piperine derivatives, $GABA_A$ receptor, snapshot PK study, UHPLC-MS/MS.

References:
[1] Zaugg et al. J Nat Prod 2010; 2: 185-91
[2] Schöffmann A. et al. J Med Chem 2014
[3] Li C et al. Drug Discov Today 2013; 18: 71.78

P-4

Validation of UHPLC-MS/MS Methods for Quantification of Kaempferol and 4-Hydroxy-phenylacetic Acid in Rat Plasma and Application to Pharmacokinetic Studies

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Introduction: Kaempferol is a major flavonoid in the human diet and in medicinal plants, and has been shown to possess antidepressant properties when administered orally. The compound undergoes transformation into 4-hydroxyphenylacetic acid (4-HPAA) by the intestinal microflora [1]. However, the fate of the metabolite and its pharmacological effects in the body are largely unknown [2].
Aim: Validation of UHPLC-MS/MS methods for quantification of kaempferol and its major metabolite 4-HPAA in Li-heparin plasma of male Sprague Dawley rats to support pharmacokinetic studies after intravenous (i.v.) administration.
Methods: Both UHPLC-MS/MS methods were developed and validated according to international guidance [3]. $^{13}C_{15}$ kaempferol was

selected as internal standard (IS) for quantitation of kaempferol, and 4-HPAA-d2 was selected as IS for quantification of 4-HPAA. To avoid matrix effects, plasma samples were subjected to phospholipid removal plate from Waters (Ostro 96-well plate) for kaempferol method whereas protein precipitation of bovine serum albumin (BSA) as surrogate matrix devoid of the target analyte was used for the 4-HPAA method.
Kaempferol and 4-HPAA were administered in multiple doses (1-2-4 mg/kg b.wt. i.v) to rats. Blood samples taken from 0 to 12 h were analyzed by non-compartmental analysis using WinNonlin software (version 5.2.1, Pharsight, St. Louis, MO, USA).
Results: Both ranges were from 20.0 to 2000 ng/mL, and the calibration curves were fitted with a quadratic curve with 1/X as weighing factor. Carryover was within acceptance criteria (below 20% for analyte and below 5% for IS). Both analytes were stable in biological samples during sample collection and handling, during 1 month storage below -65°C and after 3 freeze and thaw cycles.
After i.v. application of 4 mg/kg, the clearance (CL) of kaempferol was high 4.06 ± 0.18 L/h/kg, and the volume of distribution (V_d) was 0.4 ± 0.03 L/kg. The terminal elimination rate constant (k_e) and the terminal half-life ($t_{½}$) were 10.35 ± 0.41 h^{-1} and 4.2 ± 0.003 min, respectively. The pharmacokinetic parameters of 4-HPAA were CL = 1.04 ± 0.08 L/h/kg, V_d = 1.47 ± 0.48 L/kg, k_e = 1.3 ± 0.35 h^{-1} and $t_{½}$ = 1 ± 0.32 h.
Conclusions: Both bioanalytical methods are specific, selective, precise, accurate, and capable to produce reliable results. The high CL, resulting in the high k_e, and the short $t_{1/2}$ suggest that kaempferol undergoes intensive metabolism. The relatively high V_d of its metabolite compared to the rat total body water content demonstrates a potential binding in a peripheral site, which allows 4-HPAA to exert a pharmacological effect [2].

Keywords: UHPLC-MS/MS, kaempferol, 4-HPAA, pharmacokinetics, non-compartmental analysis.

References:
[1] Blaut M et al. Int J Vitam Nutr Res 2003; 2: 79-87.
[2] Vissiennon C et al. J Nutr Biochem 2012; 7: 733-40.
[3] Food and Drug Administration (2001) Guidance for Industry: Bioanalytical Method Validation, Food and Drug Administration, May 2001.

P-5

Pharmacokinetics and *In Vitro* Blood-Brain Barrier Screening of an Anti-inflammatory Indolinone from *Isatis tinctoria*

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Introduction: Previously, we identified *(E,Z)*-3-(4-hydroxy-3,5-dimethoxybenzylidene)-indolin-2-one (indolinone, Fig. 1) from woad *(Isatis tinctoria* L., Brassicaceae) as a compound possessing histamine release inhibitory and anti-inflammatory properties [1].

Aims: Investigation of indolinone pharmacokinetics and *in vitro* blood-brain barrier (BBB) permeation to further evaluate its potential as a new chemical entity.
Methods: A bioavailability study was carried out in male Sprague Dawley rats (2 mg/kg b.wt. i.v.) with blood sampling up to 12 h after injection [2]. The ability of indolinone (5 µM) to cross the BBB was assessed in human and animal *in vitro* BBB models. P-glycoprotein (P-gp) interaction for indolinone was studied with the aid of a calcein uptake assay, and by calculation of the efflux ratio (ER) from bidirectional permeability assays. Both LC-MS/MS quantification methods for indolinone in lithium heparin rat plasma and Ringer HEPES buffer were validated according to international guidance [3].
Results: A short half-life $t_{1/2}$ (4.30 ± 0.14 min) and a relatively high clearance CL (3.83 ± 1.46 L/h/kg) were found in rat plasma. In the human BBB model (hBMEC cell line), the apparent permeability coefficient from apical (A) to basolateral (B) ($P_{app\ A\rightarrow B}$), and the P_{app} from B to A ($P_{app\ B\rightarrow A}$) were 19.2 ± 0.485 x 10^{-6} cm/s and 21.7 ± 0.326 x 10^{-6} cm/s, respectively. The calculated ER thus was 1.13. In the primary rat/bovine BBB *in vitro* co-culture model, the $P_{app\ A\rightarrow B}$ was 27.1 ± 1.67 x 10^{-6} cm/s. No significant impact on calcein assay was observed with indolinone (5-50-500 µM) in comparison to verapamil.

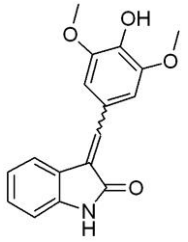

Figure 1 (E,Z)-3-(4-hydroxy-3,5-dimethoxybenzylidene)-indolin-2-one (indolinone)

Conclusion: The low $t_{1/2}$ and high CL demonstrate the need of rat urine collection for next PK studies. The data obtained with both BBB models showed a high BBB permeation potential of indolinone. The ER below 2 indicates that indolinone is not subject to active mediated transport mechanism. The calcein assay showed that indolinone is neither a P-gp substrate nor a P-gp inhibitor.

Keywords: *Isatis tinctoria,* indolinone, pharmacokinetics, blood-brain barrier (BBB), LC-MS/MS.

References:
[1] Kiefer S et al. Eur J Pharm Sci 2010; 40:143-147.
[2] Oufir M et al. J Chrom B 2012; 902: 27-34.
[3] Guidance for Industry: Bioanalytical Method Validation, US Food and Drug Administration (FDA), Center for Drug Evaluation and Research, May 2001.

P-6

Incidence of and Risk Factors for Severe Hypoglycemia in Treated Type 2 Diabetes Mellitus Patients in the United Kingdom

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Introduction: Several risk factors are known for severe hypoglycemia (SH) in patients with type 2 diabetes mellitus (T2DM), but data on incidence rates (IRs) of and risk factors for SH from a "real world" setting are scarce.

Aims: To assess the proportion of patients with, and IRs of recorded SH, and to further characterize potential risk factors for SH.

Methods: We performed a retrospective cohort study in T2DM patients derived from the UK-based General Practice Research Database and assessed IRs (with 95% confidence intervals [95% CIs]) of incident SH per 10,000 person-years (PYs). In a nested case-control analysis we compared cases (patients with recorded SH) with matched (1:10) controls without hypoglycemia during follow-up. Using conditional logistic regression models we computed adjusted odds ratios (ORs) with 95% CIs of SH in association with various potential risk factors of interest.

Results: Of 130,761 patients with T2DM (mean age 61.7 ± 13.0 years), 690 (0.5%) were identified with an incident SH event during follow-up. The estimated IR overall was 12.0 (95% CI 11.1-12.9) per 10,000 PYs. IRs were markedly higher in insulin users (49.6 [44.1-55.9] per 10,000 PYs) than in patients not using insulin (8.0 [7.3-8.8] per 10,000 PYs). Sex and cardiovascular disease did not alter the SH risk. Risk factors associated with an increased SH risk included age (adjusted [adj.] OR 2.3 [95% CI 1.7-3.1] in patients ≥ 75 years vs. those aged 20-59 years), cognitive impairment/dementia (adj. OR 2.0 [1.4-2.9]), renal impairment (adj. OR 1.3 (1.0-1.7), current sulfonylurea (SU) use (adj. OR 4.5 [3.5-5.6]), and current insulin use (adj. OR 11.8 [9.0-15.5); current metformin use was associated with a slightly decreased SH risk (adj. OR 0.8 [0.6-1.0]).

Conclusions: In this large UK cohort of T2DM patients, age, cognitive impairment/dementia, renal impairment and current use of insulin or SU significantly increased, while current metformin use decreased the risk of developing SH. Overall, these findings are in concordance with results from previous studies.

Keywords: Type 2 diabetes mellitus, severe hypoglycemia, general practice research database.

P-7

Validation of UHPLC-MS/MS Methods for Quantitation of Kaempferol and 4-Hydroxy-Phenylacetic Acid, and Application to *in vitro* Blood Brain Barrier and Intestinal Drug Permeability Assays

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Introduction: In mice, the flavonoid kaempferol induces anxiolytic activity after oral administration (p.o.), but not after intraperitoneal (i.p.) injection. However, i.p. application of its major metabolite 4-hydroxy-phenylacetic acid (4-HPAA), naturally formed by the intestinal microflora, induces behavioral changes [1]. The systemic distribution and brain penetration of flavonoids and their metabolites are not fully understood, and reliable quantification methods are needed.

Aims: To evaluate the ability of kaempferol and 4-HPAA to cross intestinal and blood brain barriers (BBB), we screened these compounds in human and animal cell-based models using validated UHPLC-MS/MS methods for reliable quantification in buffers.

Methods: UHPLC-MS/MS methods were developed and validated according to international guidelines [2, 3]. $^{13}C_{15}$-kaempferol served as internal standard (IS) for quantitation of kaempferol, and vanillic acid was selected as IS for the determination of 4-HPAA. BBB transport studies using Ringer HEPES buffer as transport medium were performed with a human *in vitro* BBB model using immortalized hBMEC cell line and a rat triple co-culture model using primary brain endothelial cells, pericytes and astrocytes, whereas human intestinal permeability assays were performed with human Caco-2 cell line using HBSS buffer as transport medium.

Results: Range for both analytes was 20.0 to 2000 ng/mL and the calibration curves were fitted with a quadratic curve with $1/X^2$ as weighing factor. Carryover was within acceptance criteria. Both analytes were stable in biological samples during sample collection and handling, during 2 weeks storage below -65°C, and after 2 freeze and thaw cycles. In the Caco-2 model, the apparent permeability coefficient (P_{app} A-B) of kaempferol from apical (A) to basolateral (B) compartment and from B to A side (P_{app} B-A) were 23.7 x 10^{-6} and 32.1 x 10^{-6} cm/s, while those of 4-HPAA were 2.3 x 10^{-6} and 4.0 x 10^{-6} cm/s, respectively. In the human BBB model, P_{app} A-B and P_{app} B-A of kaempferol were 37.3 x 10^{-6} and 29.1 x 10^{-6} cm/s, and those of 4-HPAA were 8.3 x 10^{-6} and 7.1 x 10^{-6} cm/s, respectively. In the rat BBB model P_{app} A-B and P_{app} B-A of kaempferol were 28.0 x 10^{-6} and 18.3 x 10^{-6} cm/s.

Conclusions: All UHPLC-MS/MS methods were specific, selective, precise, accurate, and capable to produce reliable results. The permeability studies were indicative of high intestinal and BBB permeation of kaempferol, whereas its metabolite 4-HPAA showed low permeability in all studied barrier models. Our *in vitro* data support the previous described *in vivo* CNS effects of kaempferol while the role of 4-HPAA needs to be elucidated in further studies.

Keywords: Kaempferol, 4-HPAA, method validation, BBB, Caco-2.

References:

[1] Vissiennon C et al. J Nutr Biochem 2012; 7: 733-40.
[2] Guidance for Industry: Bioanalytical Method Validation, US Food and Drug Administration (FDA), Center for Drug Evaluation and Research, May 2001.
[3] Guideline on Bioanalytical Method Validation. European Medicines Agency (EMEA/CHMP/EWP/192217/2009), London, 21 July 2011.

P-8

Validation of an *In Vitro* Cell-Based Human Blood-Brain Barrier Model by Using Reliable LC-MS/MS Quantitative Methods of Test Compounds

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Introduction: Cell-based *in vitro* blood-brain barrier (BBB) models have been widely implemented in academia and industry for early prediction of brain penetration of lead compounds. However, human models based on cell lines often lack sufficient barrier tightness. To establish an improved human *in vitro* BBB model, we have previously evaluated various immortalized cell lines with respect to their ability to provide endothelial cell layers with sufficient barrier tightness. hBMEC cell line was found to be most suited for this purpose [1].
Aims: In this study, we aimed at validating this cell line-based human *in vitro* BBB model with multiple test compounds which are known to cross the BBB to a different extent.
Methods: To determine the paracellular permeability of 10 selected test compounds in the BBB model, quantitative LC-MS/MS methods are currently being developed and validated according to current regulatory guidelines [2,3].
Results: Up to now, two positive test substances (diazepam and propranolol) were screened in the *in vitro* BBB model in parallel with the integrity marker sodium fluorescein (Na-F) which does not cross the BBB in a significant amount. Paracellular permeability coefficients (Pe) were 69.5 x 10^{-6} cm/s for diazepam, and 56.3 x 10^{-6} cm/s for propranolol. Pe values for Na-F were in the range of 5 x 10^{-6} cm/s.
Conclusions: Compared to the negative control Na-F, both diazepam and propranolol showed significantly higher Pe values, indicating a high BBB penetration. After validation of the human *in vitro* BBB model with the remaining 8 test substances, natural product derived leads with promising *in vitro* activity will be screened for their ability to pass the BBB.

Keywords: Blood-brain barrier (BBB), validation, paracellular permeability, LC-MS/MS, propranolol.

References:

[1] Eigenmann DE et al. Fluids Barriers CNS 2013; 10: 33-50.
[2] Guidance for Industry: Bioanalytical Method Validation, US Food and Drug Administration (FDA), Center for Drug Evaluation and Research, May 2001.
[3] Guideline on bioanalytical method validation. European Medicines Agency (EMEA/CHMP/EWP/192217/2009). London, 21 July 2011.

P-9

Sun Protective Behavior of Tourists Traveling to Sunny Holiday Destinations

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Introduction: The development of malignant melanoma has been associated with intense episodic sun exposure, as it typically occurs during holidays in high UV-index countries.
Aims: To investigate whether and by what means tourists traveling to holiday destinations in the tropics and subtropics seek to prepare their skin for the planned sun exposure, to examine their sun protective behavior, and to assess their sunburn rate during the vacation.
Methods: Between February and April 2013, we conducted structured face-to-face interviews among passengers waiting in the departure or the baggage claim area at the Airport Basel-Mulhouse (Switzerland/France). Passengers of at least age 18 with Caucasian skin whose holiday destination was the Caribbean (Cancun, Punta Cana), the Canary Islands (Fuerteventura, Gran Canaria, Teneriffa) or North Africa (Marrakech, Hurghada, Sharm el-Sheikh) were eligible for interviews.
Results: We completed 533 interviews with departing and 324 interviews with returning tourists. Of the departing tourists, 10.1% visited a solarium within the 4 weeks preceding the vacation, 72.2% of them with the intent of preparing their skin for the anticipated sun exposure and thereby preventing sunburn. Furthermore, 7.8% sought to prepare the skin by taking dietary supplements such as carotenoids. However, 59.5% started the supplementation less than 9 weeks before departure and were thus unlikely to benefit from any extra protection. Almost all (97.4%) departing tourists had sunscreen in their luggage. The majority used sunscreen with a medium to very high sun protection factor (SPF 15-20: 8.3%; SPF 25-30: 46.2%; SPF $\geq$ 40: 41.2%). Yet, 21.0% of sunscreen users stated to reduce the SPF on average by half after some days of sun exposure. Other sun protective items in the tourists' luggage included sunglasses (93.8%), sunhat (63.6%), and clothes with integrated UV protection (4.1%). Of the returning tourists, 79.0% nearly always applied sunscreen on sunny days, whereas only 34.0% and 38.9% usually wore a sunhat and stayed in the shade around noon, respectively. Of concern, 44.4% affirmed they suffered from sunburn during their vacation, with nearly every third sunburn was described as painful. Male gender, younger age, *not wearing a sunhat* and *only sometimes using sunscreen* were associated with increased odds of sunburn.
Conclusions: The observed sunburn rate was alarmingly high. Future skin cancer prevention programs should reveal common misconceptions in terms of preparing the skin for the sun and emphasize the avoidance of sunburns, the correct use of sunscreen as well as the importance of covering up and seeking shade.

Keywords: Melanoma, sun protection, sunburn rate, flight passenger interviews.

P-10

Development of Floating Gastroretentive Minitablet Formulations

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Introduction: Floating drug delivery systems are designed to prolong and control the residence time of drug substances in the gastric region. In comparison to single-unit dosage forms, multiple-unit floating devices (e.g. granules, pellets, minitablets) offer the possibility to reduce the risk of "all-or-nothing" gastric emptying [1]. Functionalized calcium carbonate (FCC) has been evaluated as a floatation promoting excipient in the preparation of floating tablets.
Aims: The objective of the study was to develop an optimal floating FCC-based minitablet formulation using experimental design.
Methods: The experimental design software *STAVEX* was applied to develop and optimize the floating minitablets. Thirteen different formulations were analyzed, where the percentages of the 3 selected excipients were changed. The floating minitablet mixtures were granulated with the hot melt extruder. To investigate the *in vitro* dissolution and floating behaviour, a custom-built stomach model [2] was used. The 85% caffeine release times and the tablet hardness were chosen as *STAVEX* response variables.
Results: All prepared minitablets were floating immediately and showed no floating lag time. LUBRITAB® had a positive influence on the tablet hardness and delayed the caffeine release. FCC also made the tablets harder, whereas it speeded up the drug release. POLYOX™ did not have an effect on the hardness nor on the drug release time. An optimal minitablet formulation containing 43% (w/w) LUBRITAB®, 40% (w/w) FCC, and 17% (w/w) caffeine was produced.
Conclusions: The hot melt extruder was found to be suitable for hot melt granulation. It was possible to compact floating minitablets with the prepared granules. Depending on the composition of the floating minitablet formulations, different drug release behaviours and times from 1-27 h can be achieved. Optimal floating minitablets with the desired properties, such as sufficient hardness, no floating lag time, and a complete drug release within 20 h were prepared.

Keywords: Gastric retention, floatation, multiple-unit drug delivery system, stomach model.

References:
[1] Streubel A, Siepmann J, Bodmeier R. Expert Opin Drug Deliv 2006; 3: 217-233.
[2] Eberle VA et al. Eur J Pharm Sci 2014; 58: 34-43.

P-11

Treatment of Urinary Tract Infections - Optimized Drug Delivery of FimH Antagonists

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Introduction: Urinary tract infection (UTI) is one of the most common infectious diseases and is mainly caused by uropathogenic *Escherichia coli* (UPEC). The first step of the infection, the adhesion of UPEC to urothelial cells, is mediated by FimH, a lectin located at the tip of bacterial type 1 pili. Its interaction with mannosylated glycoproteins on urothelial cells can be prevented by glycomimetics. Thus, biphenyl and indolinylphenyl α-D-mannopyranosides with nanomolar affinity offer a potential therapeutic approach for the prevention and/or treatment of UTIs.
Aims: To achieve oral availability as well as a therapeutic concentration in the bladder over an extended period of time, a number of key issues have to be fulfilled. First, sufficient solubility is a prerequisite for successful absorption. Second, to reach circulation, permeation through the intestinal mucosa is required. Third, in case of a prodrug approach, hepatic first pass metabolism is requested. Finally, to reach the therapeutic target in the bladder, renal excretion is required.
Methods: These properties can be predicted based on lipophilicity ($logD_{7.4}$), thermodynamic solubility (shake-flask method), metabolic stability (liver microsomes), and permeability (parallel artificial membrane permeability assay and Caco-2 cells assay), methods implemented in the PADMET platform of our research group.
Results: Low membrane permeability was identified as a drawback constraining oral absorption of biphenyl α-D-mannopyranosides substituted with a carboxylic acid at the terminal ring of the aglycone. With ester prodrugs the permeability problem could be solved. Furthermore, cleavage by carboxylesterase released the active principle within minutes. Nevertheless, low solubility was identified as a new drawback for the ester prodrugs and additionally for the good permeable indolinylphenyl α-D-mannopyranosides.
Conclusions: For early *in vivo* trials, the solubility issue was addressed with appropriate formulations using co-solvents, surfactants, and complexing agents.

Keywords: FimH antagonists, oral absorption, permeability, solubility, preformulations.

P-12

Novel Excipient: Functionalized Calcium Carbonate (FCC) with Lamellar Structure as a Key Factor for Unique Tablet Properties

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Introduction: In the past decade the request for new, multifunctional materials to be used in pharmaceutical development has started. Omya developed such a new excipient with unique properties, the Functionalized Calcium Carbonate (FCC). FCC is a particulate material with a size of 5-10 µm and highly developed surface and internal structures. The particles show a specific lamellar surface area of 40-80 m^2/g and a porosity of approximately 70%.
Aims: The aim of the study was to characterize the influence of FCC surface properties on the mechanical stability of tablets from pure materials and FCC-based granules with a focus on excipient application. Furthermore, we investigated the facilitated development of challenging formulations such as orally dispersible tablets (ODT) by the utilization of these unique FCC surface properties.
Methods: During the compaction study all the excipients were compacted at a compressive pressure ranging from 6 MPa to 500 MPa with a Styl'One tablet press. For the ODT development the tablets were compressed with an excenter press (Korsch EK0) to a target hardness of 100 N. Disintegration and dispersion kinetics were measured with a metal-wire basket which was attached to

the microbalance of a tensiometer. The residence time was calculated with a user-defined fitting based on the weight loss against time profile.
Results: Compaction of FCC into robust tablets needed lower compressive pressure compared to the other excipients such as microcrystalline cellulose (MCC), mannitol, and calcium carbonate. The properties of high tensile strength could be explained by a mechanistic model based on contact surfaces of FCC particles. Furthermore, the lamellar structure was responsible for a capillary suction effect during the water absorption into a tablet, resulting in a very fast tablet disintegration time of approximately 10 s at a tablet hardness of 100 N.
Conclusions: FCC showed similar compactability and compressibility properties as for MCC. The surface properties of FCC had a very strong positive effect on the mechanical stability of FCC-based compacts. Furthermore, ODTs with sufficient tablet hardness and a fast disintegration time were obtained by direct compression. The comparison of developed ODT formulation with the market product risperidone oro has demonstrated excellent performance of FCC as a new enabling excipient.

Keywords: Contact surface, tensile strength, porosity, disintegration.

P-13

Evaluation of the Chick Embryo as an *In Vivo* Test System for Radiopharmaceuticals

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Introduction: The chick embryo and its chorioallantoic membrane (CAM) are widely used in different fields of biomedical research [1]. In radiopharmaceutical sciences it has been reported that this *in vivo* model presents a suitable test system for studying the distribution, uptake and kinetics of radiotracers by micro-positron emission tomography (PET) as demonstrated for [^{18}F]fluoride [2]. Moreover, it was recently shown that xenografts grown on the CAM can be used to explore the tumor uptake of novel radiotracers by PET imaging [3].
Aims: The aim of this study was to investigate if the chick embryo is a feasible model for evaluating the biodistribution and *in vivo* stability of radiopharmaceuticals by single photon emission tomography (SPECT) and PET imaging. For this purpose the behavior of various radiopharmaceuticals was compared in the chick embryo and the mouse. Amongst others the renal radiotracer ^{99m}Tc-DMSA, the bone radiotracer ^{99m}Tc-MDP and ^{18}F-fallypride, which targets the dopamine D2 receptor in the brain, were explored in these two species.
Methods: Fertilized chicken eggs were cultivated for 72 h at 37°C before the egg shell was cracked for *ex ovo* cultivation of the embryos. After 17-19 d ~60 MBq of ^{99m}Tc-DMSA, ~50 MBq of ^{99m}Tc-MDP or ~10 MBq of ^{18}F-fallypride were injected intravenously into a blood vessel of the CAM or intraperitoneally. The chick embryos were euthanized in liquid nitrogen 1.5-5 h p.i. followed by SPECT or PET acquisitions. SPECT and PET scans lasted between 5 and 15 min depending on the amount of radioactivity left in the organism at scan start. All results were compared with those obtained with the same radiotracers in the mouse model.
Results: SPECT/CT images of the chick embryo after the injection of ^{99m}Tc-DMSA clearly showed uptake of radioactivity in the kidneys. ^{99m}Tc-DMSA is known to have the same biodistribution in mice which was confirmed in our current studies. ^{99m}Tc-MDP accumulated in the bones of chick embryos as well as in the skeleton of mice as demonstrated in our SPECT/CT studies. PET scans revealed uptake of radioactivity in the intestine of the chick embryo after the injection of ^{18}F-fally-pride. Accumulation of ^{18}F-fallypride in the dopaminergic regions of the chick embryo brain was difficult to determine due to its small size. In the mouse ^{18}F-fallypride accumulated also in the intestine where dopamine receptors are expressed. Moreover, ^{18}F-fallypride bound to the D2 receptors in the mouse brain. Additionally, in both species accumulation of radioactivity was observed in the bones. This indicated *in vivo* defluorination since free [^{18}F]fluoride is known to be entrapped in the bones as confirmed in our studies for both species.
Conclusions: In this work we demonstrated that the tested radiopharmaceuticals have compa-rable *in vivo* behaviors in chick embryos and mice. Based on this data, we conclude that the chick embryo could be a reliable and cost-effective alternative to the mouse for the first evaluation of novel radiopharmaceuticals. These promising results warrant further investigations using additional radiopharmaceuticals.

Keywords: Chick embryo, PET, SPECT, radiopharmaceutical, *in vivo* behavior.

References:
[1] Rashidi H, Sottile V. BioEssays 2009; 3: 459-65.
[2] Würbach L, Heidrich A, Opfermann T. Mol Imaging Biol 2012; 14: 688-98.
[3] Warnock G, Turtoi A, Blomme A. J Nucl Med 2013; 54: 1782-8.

P-14

Photoprotective and Antioxidative Constituents of an Alpine Scabiosa Species

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Introduction: UV-related damage is one of the main factors leading to skin cancer. The cosmetic industry puts intensive research efforts into the discovery of new ingredients with photoprotective and antioxidative properties, as it has been recognized that high sun protection factors alone are not sufficient to block UV-induced effects. Switzerland possesses a rich and diverse alpine flora which represents an attractive source of new ingredients with skin-protective effects, as alpine plants typically produce UV-protective secondary metabolites in response to high UV exposure.
Aims: The objective of this study is to develop a cosmetic ingredient based on alpine plants to protect skin against UV-induced damages.
Methods: More than 30 ethanolic extracts from alpine plants were tested for protecting activity against cyclobutane pyrimidine dimer (CPD) formation in keratinocytes, and for DPPH radical scavenging properties. A *Scabiosa* species was identified as a promising candidate in both assays. It was selected for comprehensive investigation of its metabolite profile and characterization of the bioactive constituents. Dried aerial parts were extracted successively by dichloromethane and methanol. Fractionation of the methanolic extract was achieved by a combination of different chromatographic methods, such as column chromatography on Sephadex LH-20, and preparative and semipreparative HPLC.
Results: Targeted purification afforded 14 compounds including flavonoids (luteolin and isoorientin O- and C-glucosides), caffeic

acid derivatives, and iridoid and secoiridoid glucosides. These metabolites accounted for most peaks detected in the HPLC-UV/ESIMS profile of the crude extract. Flavone glucosides with catechol moieties and caffeoylquinic acid derivatives were identified as responsible for the DPPH scavenging activity. Protection against CPD formation could be assigned to fractions containing caffeoylquinic acid derivatives. A compound consisting of an ester of menthiafolic acid with secologanol was identified as a new secondary metabolite.
Conclusions: The methanolic extract of *Scabiosa* sp. and some of its constituents exhibit signifi-cant DNA protection and DPPH radical scavenging properties. The extract can be considered as a promising candidate for the development of a new ingredient in UV-protective cosmetic formulations.

Keywords: Alpine plants, free radical scavenging, DNA protection, cosmetics.

P-15

The Risk of Incident Rosacea Associated with Psychiatric Diseases and Psychotropic Drug Therapy

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Introduction: Rosacea is a chronic skin disease of unclear origin. Despite scarce evidence, the skin disease has been linked to emotional stress and to anxiety. A large case-control study reported an increased co-occurrence of depression and rosacea. We are not aware of any studies assessing a link between rosacea and schizophrenia or use of antidepressant or antipsychotic drugs.
Aims: To analyze the association between psychiatric diseases and psychotropic drugs and the risk of incident rosacea.
Methods: We conducted a matched (1:1) case-control analysis using the UK-based General Practice Research Database. We included incident rosacea cases diagnosed between 1995 and 2009, and compared the prevalence of psychiatric diseases and the exposure to antidepressants and antipsychotics prior to the index date between cases and controls.
Results: We included 60,042 rosacea patients and the same number of controls. Depression was diagnosed in 10,413 cases (17.3%) prior to the index date, resulting in an adjusted odds ratio (OR) of 1.17 (95% CI 1.13-1.21). Other affective disorders (i.e. bipolar- / manic disorders) revealed an adj. OR of 1.16 (95% CI 1.08-1.25). With regard to antidepressant drugs, selective serotonin reuptake inhibitors and monoamine reuptake inhibitors did not affect the risk estimate, but exposure to lithium revealed significantly decreased ORs, namely in current long-term users (OR 0.59, 95% CI 0.40-0.87, for users of 40+ prescriptions with a last recorded prescription <180 days). This effect was similar in patients with or without a concomitant schizophrenia diagnosis. We observed a significantly decreased adjusted OR of 0.71 (95% CI 0.60-0.84) for patients with schizophrenia. Stratification of these patients according to their drug treatment revealed decreased ORs across all strata. Neurotic, stress-related, and somatoform disorders (NSSD) revealed an OR around unity.
Conclusions: Contrarily to previous findings, depression and NSSD were not associated with rosacea. On the other hand, our results suggest a decreased rosacea risk in patients with schizophrenia, independently of antipsychotic drug treatment. Furthermore, we observed decreased ORs in patients on lithium, irrespective of the underlying psychiatric diagnosis. To our knowledge, this is the first description of a potential effect of lithium on rosacea.

Keywords: Rosacea, psychiatric diseases, psychotropic drugs, pharmacoepidemiology, general practice research database.

P-16

Evaluation of a Potent FimH-Antagonist for Urinary Tract Infection Treatment

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Introduction: Anti-adhesion therapy, a new therapeutic approach to treat infectious diseases, avoids killing of the pathogens, reducing selective pressure and resistance development. In urinary tract infections (UTI), uropathogenic *E. coli* (UPEC) use FimH-adhesins located on the tip of type 1 pili for the attachment to urothelial epithelial cells of the host. This adhesion process is a required prelude for invasion, colonisation and biofilm formation. FimH-antagonists provide a novel strategy for preventing urinary tract infection (UTI) by blocking the interaction of FimH with its urothelial ligand.
Aims: In this study, possible therapeutic applications for FimH-antagonists within UTI should be assessd. Shown are the results of a potent biphenyl α-D-mannoside derivative, which proofed beneficial for preventing and treating UTI.
Methods: An α-D-mannoside derivative was evaluated as FimH-antagonist in an *in vitro* infection model with urothelial cells, allowing the determination of the minimal anti-adhesive concentration (MAC) by flow cytometry. Moreover, the compound was orally administered to female C3H/HeN mice for pharmacokinetic (PK) studies and for its potential to prevent infection with UTI89 in the UTI mouse model. Furthermore, the compound was tested in a prevention study up to 24 h to assess basic PK/PD and possible treatment regimens. Subsequently, a combined prevention/ treatment set-up and a combination therapy with ciprofloxacin were conducted.
Results: The compound showed high *in vitro* activity (MAC: 0.09 µg/ml) and a favourable PK profile, with a fraction absorbed in urine between 45-60%. Furthermore, it resulted in a bacterial reduction in the bladder upon infection with UTI89 of more than 3 Log_{10} units after 3 h, even beating the effect of ciprofloxacin with a reduction of 2.4 Log_{10} units. Then, the FimH-antagonist was studied for the treatment of cystitis, combining preventive and therapeutic doses, resulting in a 1.4 Log_{10} unit decrease of CFU/ml in the bladder after 9 h. Finally, the FimH-antagonist was also tested in combination with ciprofloxacin in various set-ups and proofed beneficial for possible applications in combination therapies.
Conclusion: The biphenyl α-D-mannoside derivative proofed effective as FimH-antagonist in preventing and treating cystitis in different treatment set-ups. FimH-antagonists could provide a new option for treating UTI, one of the most common bacterial infections worldwide, reducing the use of antibiotics and therefore, help to slow down resistance development.

Keywords: Urinary tract infection, anti-adhesives, FimH-antagonists.

P-17

Screening of Potential Endocrine Disrupting Chemicals by Metabolomics Analysis of Adrenal H295R Cell Supernatants

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Introduction: Adrenal steroidogenesis involves the enzymatic conversion of cholesterol to gluco-corticoids *(e.g.* cortisol), mineralocorticoids *(e.g.* aldosterone) and androgen precursors *(e.g.* dehydroepiandrosterone). These pathways are tightly regulated and disturbances can result in an altered immune response, metabolic disease or developmental dysfunctions. Therefore, it is important to identify xenobiotics interfering with adrenal steroidogenesis and to understand their molecular mechanism of action. Recent evidence suggested that triclocarban, an antibacterial agent used in personal care products such as soaps and deodorants, is a potential endocrine disruptor.
Aims: Establishment of a metabolomics-based screening strategy to evaluate potential endocrine disruptors. Triclocarban will be used as a test substance for this approach.
Methods: H295R cells were treated with the potential endocrine disruptor triclocarban (0.5 - 10 µM) following an assay described in the OECD Guidelines (Test No. 456: H295R Steroidogenesis Assay). Untargeted UHPLC-QTOF MS acquisition was performed on the extracted cell supernatant. Data extraction was performed using Progenesis QI. Steroid-like compounds were extracted according to their *m/z* using steroids lists obtained from publicly available databases (HMDB and LipidMaps). Further multivariate analyses (Orthogonal Partial Least Square – Discriminant Analysis (OPLS-DA) and correlation analyses) were used to further extract and rank disturbed steroids.
Results: Based on the dose-response data and multivariate analyses, a group of 50 steroid-like compounds was selected as predictive of triclocarban concentration. Using correlation analysis, these steroids were then grouped according to the effect of triclocarban to reduce their concentration; a first group (7 compounds) representing the steroids being highly sensitive towards triclocarban concentration increase, a second one (33 compounds) gathering moderately sensitive compounds, and a third one (7 compounds) gathering less sensitive steroids. Amongst these steroids, 7 were identified and quantified; pregnenolone, 11-deoxycorticosterone and progesterone were "highly sensitive", 11-dehydrocortico-sterone, aldosterone and dehydroepiandrosterone sulfate were "moderately sensitive", and cortisone showed "low sensitivity" towards triclocarban treatment. These data suggest a perturbation in the early steps of adrenal steroidogenesis by triclocarban.
Conclusions: We developed a UHPLC- High Resolution MS qualitative and quantitative platform for the study of endocrine disruptors, which can be used as a screening strategy to assess the safety of xenobiotics.

Keywords: Endocrine disrupting chemical, adrenal gland, triclocarban, H295R, steroidogenesis.

P-18

Quantification of Bufadienolides in Bryophyllum Pinnatum Leaves and Manufactured Products

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Introduction: *Bryophyllum pinnatum* (Crassulaceae) is a succulent perennial plant native to Madagascar. It is used in anthroposophical medicine to treat psychiatric disorders, and as a tocolytic agent to prevent premature labour. Besides flavonoids, the plant is known to contain bufadienolides, which reportedly possess sedative and positive inotropic properties, as well as central nervous system related activities.
Aims: Quantification of bufadienolides in various batches of *B. pinnatum* leaves and press juices in the context of safety assessment.
Methods: A validated UHPLC-ESI-MS/MS method was used for quantification of the main bufa-dienolides bryophyllin A, bersaldegenin-1-acetate, bersaldegenin-3-acetate, and bersaldegenin-1,3,5-orthoacetate. Separation was performed on a Kinetex XB-C18 column with a gradient of MeCN (0.05% HCOOH) in H_2O containing 10 mM ammonium formate and 0.05% HCOOH. Leaves were extracted with EtOH by accelerated solvent extraction (ASE). Press juices were dried and re-dissolved in DMSO or, alternatively, extracted by partitioning with EtOAc.
Results: The content of the 4 bufadienolides was 1.18, 0.99, 2.08 and 0.94 mg/100g dry weight (DW), respectively, in a representative batch of leaves from plants grown in Germany. With 7.75, 1.45, 4.89 and 5.17 mg/100 g DW, respectively, the contents were significantly higher in plants grown in Brazil. The total amount of bufadienolides was 1.71 and 0.59 mg/100 ml in press juices obtained from plants cultivated in Brazil and Germany, respectively. Although there were some differences in the contents in juices prepared from plants harvested at different dates, no correlation was observed between the time of harvest and the content.
Conclusion: This study provides for the first time reliable data on the content of bufadienolides in *B. pinnatum.* While the harvest time appears to have no predictable influence on the bufadienolide content, plants cultivated in Brazil were consistently found to contain significantly more bufadienolides than those grown in Germany. Interestingly, in all single plants investigated, the content of bufadienolides was markedly higher in young leaves.

Keywords: *Bryophyllum pinnatum,* bufadienolides, UHPLC-MS/MS.

P-19

Determination of Free Phenytoin Blood Concentrations in Patients: Measured Versus Calculated Serum Levels

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Introduction: Total drug concentration in blood is routinely determined in difficult-to-dose drugs for the therapeutic drug monitoring (TDM). To adapt correct dosing in patients with changing drug-binding proteins like albumin thereby requires the knowledge of the free fraction of highly protein-bound drugs. Phenytoin (PHT), an antiepileptic critical-dose drug, needs dose individualization in patients because of its non-linear pharmacokinetics, the >90% albumin binding, a slow dose-dependent elimination, and a narrow therapeutic index. Therefore, PHT TDM is mandatory in emergency patients requiring rapid intravenous loading and subsequent correct dosing as shown in a study comparing Bayesian forecasting with conventional dosing in a tertiary care hospital [1].
Aims: The aim of the study is to evaluate the utility of the Sheiner-Tozer equation for the free serum PHT level assessment in a hospital setting. We selected patients with low serum albumin < 35 g/L [1] in whom the total and the free serum PHT concentrations were measured and compared the measured free PHT levels with the calculated ones.
Methods: 23 adult patients with a hypalbuminaemia could be selected from a total of >2500 patients [1]. They were subdivided in 2 groups: the very low albumin (alb < 25 g/L; n=12) and the low albumin group (35 > alb ≥ 25 g/L; n=11). Albumin and total PHT (dphT), determined with a routine EMIT lab assay, were analyzed in the central lab of the Kantonsspital Aarau. The free PHT (dphEF) levels were measured in the supernatant after ultracentrifugation (30 kD cutoff, 20 min centrifugation time), with a HPLC method in a specialized lab for antiepileptics (Epi Klinik Zürich). The Sheiner-Tozer equation was used to calculate the free PHT from dphT [µg/ml] and the albumin concentration [g/100 mL]:

$$\text{dphEF} = \frac{\text{dphT} \times 0.1}{a0.9 \times \frac{\text{Albumin [g/100mL]}}{4.4} + 0.1} \text{ [µg/mL]}$$

The calculated and the measured free PHT fraction were compared using Sperman Rho (non-parametric) statistics together with mean, median, and SD calculations.
Results: The two methods correlated very well (Sperman's Rho 0.907, p=0.000; n=23) and showed no significant differences. The differences between measured and calculated values were < 6.5%. The results were comparable in the low and the very low albumin group with a max. deviation between the measured and the estimated free fraction of < 6.39% (SD = 1.4; median = 3.63) and < 6.17% (SD = 1.88; median = 2.80), respectively.
Conclusion: From this study we conclude that the free PHT serum concentration can be calculated by the Sheiner-Tozer equation in hospitalized patients and hypalbuminaemia (19-35 g/L) with the necessary precision. Thus, this method represents an useful, quick, and almost free of cost bedside approach.

Keywords: Free phenytoin serum concentrations, Sheiner-Tozer equation.

Reference:
[1] Tobler A, Mühlebach S. Int J Clin Pharm 2013; 35: 790-7.

P-20

Aurones as Histone Deacetylase Inhibitors: Identification of Key Features

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Introduction: HDAC inhibition has emerged as an interesting therapeutic strategy for the treat-ment of various disorders such as cancer and neurodegenerative diseases. Clinical data demonstrated that HDAC inhibitors currently in use cause numerous side effects. Recent results suggest that inhibition of specific HDACs might be helpful to discover more effective inhibitors with reduced side effects. However, more data are required to understand which type of inhibitor is to be pre-ferred.
Aims: To search for HDAC inhibitors and identify structural features important for isoform specificity.
Methods: A total of 22 natural and synthetic flavonoids (2 chalcones and 20 aurones) were tested for their HDAC inhibitory activity using fluorimetric and bioluminescent resonance energy transfer technology (BRET)-based assay. The inhibitory specificity was assessed on HDACs 1, 2 and 6. In order to rationalize the results coming from the enzymatic assays, the 4 most active aurones were docked into the catalytic pocket of representative isoforms from class I (HDAC2) and class II (HDAC6).
Results: Four aurones were considered active in both assays and showed IC_{50} values below 20 µM in the enzymatic assay. Molecular modelling revealed that the presence and position of hydroxyl groups were responsible for good compound orientation within the isoenzyme catalytic site and zinc chelation.
Conclusion: To the best of our knowledge, our study is the first showing HDAC inhibition by aurones and these data could lead to the development of more selective and potent HDAC inhibitors.

Keywords: HDAC, structure activity relationship, natural products and analogs, aurone.

P-21

Benzodiazepine Use and Risk of Developing Alzheimer's Disease or Vascular Dementia

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Introduction: Benzodiazepines are widely used for the treatment of insomnia or anxiety. While their short-term (side-)effects on memory and cognition are well-known, there is an ongoing debate on

whether long-term use of benzodiazepines is associated with cognitive decline or dementia. Additionally, risk estimates for specific dementia subtypes such as Alzheimer's disease (AD) or vascular dementia (VD) are largely lacking.
Aims: The aim of this study was to explore the association between benzodiazepine use and the risk of developing AD or VD.
Methods: We conducted a case-control analysis using the UK-based Clinical Practice Research Datalink (CPRD, formerly known as the General Practice Research Database [GPRD]). The study population consisted of patients aged ≥65 years with newly diagnosed AD or VD between 1998 and 2013, and a comparison group of dementia-free patients matched 1:1 to AD or VD patients on age, gender, general practice, calendar time, and number of years of recorded history in the database. We used conditional logistic regression to calculate odds ratios (ORs) with 95% confidence intervals (CIs) of developing AD or VD in relation to previous exposure to benzodiazepines, stratified by duration of use, type of benzodiazepine (i.e. classical benzodiazepine vs. benzodiazepine-related drug) and type based on the estimated duration of action (ultra-short- vs. short to intermediate- or long-acting benzo-diazepines). Potential confounders were tested in bivariate analysis and results finally adjusted for body mass index (BMI), smoking, and use of antidepressants.
Results: We identified 17,644 patients with AD, 11,062 patients with VD, and 28,706 matched comparison subjects without dementia. In comparison to non-use, long-term use (≥100 prescriptions) of benzodiazepines was associated with a reduced risk of developing AD (adjusted OR 0.74, 95% CI 0.65–0.84) or VD (adjusted OR 0.86, 95% CI 0.73–1.01), although statistical significance was only reached for the former association and there was no trend with increasing number of prescriptions. Further stratification revealed that this effect was only true for classical benzodiazepines and regarding the risk of AD – only for short to intermediate-acting benzodiazepines (adjusted OR 0.78, 95% CI 0.63–0.97).
Conclusions: Long-term use of benzodiazepines was not associated with an increased risk of developing AD or VD. To the contrary, there was a suggestion of a slightly reduced risk of developing AD in long-term users of classical benzodiazepines with a short to intermediate duration of action. This may be a true effect or explained by residual confounding.

Keywords: Benzodiazepines, Alzheimer's disease, vascular dementia.

P-22

Toll-like Receptor 2 Regulates the Barrier Function of Human Bronchial Epithelial Monolayers Through Atypical Protein Kinase C Zeta, and an Increase in Expression of Claudin-1

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Introduction: The airway epithelium acts as a crucial physical and immunological barrier against several inhaled antigens and allergens. Toll-like receptor (TLR) 2 has been shown to enhance the tight junction associated epithelial barrier function of human intestinal epithelial cells.
Aim: To investigate the regulation of TLR2 in maintaining epithelial barrier integrity in human bronchial epithelial cells (Calu-3).
Methods: Transepithelial electrical resistance (TEER) and paracellular flux experiments were used in parallel to RT-PCR, Western blotting and immunofluorescence experiments to study the tight junctional regulation by TLR2. Inhibitor assays were applied to elucidate the mechanism of tight junctional regulation by TLR2 ligands.
Results: Activation of TLR2 by its ligands, Pam3CysSK4 and Peptidoglycan showed a concen-tration-dependent increase in epithelial barrier function, as measured by TEER. This was confir-med by a decrease in paracellular flux of fluorescein sodium. This TLR2 induced increase in TEER was significantly reduced by pretreatment with polyclonal anti-human TLR2-neutralizing antibody. TLR2 stimulation in Calu-3 cell monolayers resulted in an increased expression of the tight junction proteins claudin-1 and ZO-1, and a decreased expression of occludin, at both the mRNA and protein levels. A pseudosubstrate inhibitor to PKCζ significantly prevented the TLR2 mediated increase in barrier function. It also prevented the increase in claudin-1 in a concentration-depen-dent manner up to 1 μM. TLR2 stimulation led to an increase in phosphorylation of atypical PKCζ, which was prevented by the pseudosubstrate inhibitor in a concentration-dependent manner.
Conclusions: Taken together, our observations support a model whereby increased tight junction barrier function induced by activation of TLR2 occurs through increased expression of claudin-1, and through modulation of PKCζ activity.

Keywords: Calu-3, tight junctions, toll-like receptor, barrier function, protein kinase C.

P-23

An Adjuvanted Nanoparticle-based DNA Vaccine Simultaneously Enhances Immune Responses Against *Mycobacterium tuberculosis*

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Introduction: The development of new vaccine strategies using genetic material of the infectious agents is essential in the fight against tuberculosis. The optimal composition of a new generation vaccine will be comprised of a recombinant gene or antigen and different types of adjuvants to enhance its immunogenicity.
Aims: The aim of this study was to increase immunogenicity of a nanoparticle (NP) based DNA vaccine against *Mycobacterium tuberculosis* (MTb). The DNA plasmid (pDNA) encoding MTb antigen 85A also contains unmethylated CpG sequences known to activate Toll-like receptor 9 (TLR9). Subsequently, muramyl dipeptide (MDP) targeting NOD-like receptor 2 (NLR2) was added as a second immuno-stimulator to the pDNA-NP conjugates to investigate potential immuno-potentiating effects *in vitro* and *in vivo*.
Methods: Trimethyl chitosan (TMC) nanoparticle formation was the result of polymer-polymer interaction of the oppositely charged polyelectrolytes TMC and chondroitin sulfate. *In vitro* RAW 264.7 murine macrophages were stimulated with pDNA and MDP loaded NPs and the release of proinflammatory cytokine TNF-α determined by ELISA. By having applied both immune receptor ligands in one formulation a potential synergistic enhancement of the immune responses was sought to be proven, as well as the dependency of this effect on NLR2 activation. TNF-α induction was determined by ELISA before and after NLR2 inhibition by gefitinib, a RIP2 tyrosine phosphorylation inhibitor. *In vivo* the potential to stimulate systemic immune responses by pDNA-NP conjugates and the adjuvant effect of these vaccine formulations was evaluated in C57BL/6 mice

following intramuscular administration. Cellular immune response was studied by restimu-lation of spleen derived T-cells with Ag85A protein and detection of IFN-γ secretion by ELISPOT.
Results: When combining both ligands in one formulation a significant increase was observed compared to nanocarriers decorated with only one stimulatory agent. This might indicate that MDP has an influence on synergistically enhancing immune responses of pDNA-NP conjugates. T-cell analysis of mouse spleen cells showed the same trend. By blocking the NLR pathway in RAW264.7 macrophages with gefitinib, TNF-α induction in macrophages was partly blocked. The cytokine release was reduced to 79% for ligands applied in solution and 30% for TMC-NP-conjugates compared to untreated cells. The cytokine levels measured after this treatment account therefore solely for the effect of pDNA-NP conjugates and show the dependency on MDP to increase their immunogenicity.
Conclusions: This study demonstrated that NOD ligand containing NPs decorated with TLR-9 ligand pDNA very significantly increased pro-inflammatory cytokine release from murine macro-phages in a synergistic fashion. *In vivo,* T-cell activation by pDNA-NP conjugates was shown after vaccination compared to naïve mice. In accordance with *in vitro* data, MDP showed a very significant immunopotentiating effect compared to non-vaccinated mice.

Keywords: DNA vaccines, adjuvants, chitosan nanoparticles, Toll-like receptor, cell-mediated immunity.

P-24

Self-Assembling Chitosan Hydrogel via Michael Addition Reaction

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Introduction: Chitosan is a natural polymer derived from chitin with a particular chemical structure which can be easily modified. Herein, we present the synthesis of a self-assembling chitosan hydrogel via Michael addition for drug delivery. The hydrogel presents a porous structure, as proved using scanning electron microscopy, able to entrap cargo molecules. The self-assembling chitosan hydrogel is able to act as a depot and releases the cargos with the same kinetics independent of their molecular weight. Therefore it was assumed that the release of substances adsorbed to the hydrogel was mediated by diffusion.
Aim: The aim of this project is the successful modification of chitosan with sulfhydryl and male-imide moieties. Self-assembling by Michael addition is achieved by mixing the two polymers in different ratios. The hydrogel acts as a drug delivery system as confirmed by studying release kinetics.
Methods: Modification of chitosan was performed following a two-step coupling procedure. Self-assembling was achieved by mixing aqueous solutions of the two differently modified chitosan polymers. Release properties were tested in phosphate buffered saline and the amount of released cargos was quantified using UV-Vis spectroscopy. With scanning electron microscopy the porous structure of the self-assembling chitosan hydrogel was investigated.
Results and Conclusion: We achieved the synthesis of a hydrogel via Michael addition using a natural polymer as chitosan. The gel acts as a depot for various substances and cargos are released by diffusion through the porous 3D network of the gel.

Keywords: Chitosan, hydrogel, self assembly.

P-25

Overcoming the Challenges of Analytical Characterization Sensitivity of a Low Dose Active Compound in a Lipid Matrix

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Introduction: Many of the currently developed Active Pharmaceutical Ingredients (APIs) are poorly water-soluble. Solid lipid formulations have for example been developed to overcome solubility and oral bioavailability issues [1]. In these lipid matrices, the API can be in amorphous form or in solid solution. Since the presence of API crystals in the matrix can cause stability or dissolution issues, a thorough formulation characterizing is essential. However, in formulations composed of a crystalline matrix and a rather low drug amount, it can be analytically challenging to assess API crystallinity [2]. Traditional methods (DSC, XRPD, FT-IR or Raman spectroscopy) exhibit a limited sensitivity for detecting crystalline drug and new analytical strategies are required.
Aims: The aim of this work was to study lipid/API systems that could be further used in a melting process like hot-melt extrusion. β-Carotene, a low dose, poorly water-soluble API was used as model. Several analytical methods were compared regarding their ability to detect crystalline β-carotene. Moreover, a new flow-through imaging method was introduced to overcome the sampling and hence sensitivity issues encountered with the other methods.
Methods: Solid dispersions containing β-carotene and Gelucire 50/13, Geleol mono- and diglycerides were prepared by melting the physical mixtures at 125 °C in an oil bath during 5 min under a N_2 purge. The milled mixtures were analyzed by DSC at 5 °C/min and Hyper-DSC at 150 and 500 °C/min. β-Carotene crystallinity was analyzed by XRPD. Polarized light microscopy was used to qualitatively assess the presence of β-carotene crystallites in the molten mixtures. A new flow-through cross polarized imaging was employed to avoid sampling issues of classical light microscopy thereby lowering the limit of detection for crystalline drug. Moreover, a HPLC stability study was performed to control the β-carotene degradation and isomerization during the melting process.
Results: For samples containing 5% or more β-carotene, a second melting endotherm was visible in the Hyper-DSC thermograms and β-carotene crystalline peaks were visible with XRPD. With low DSC scanning rate, no β-carotene melting endotherm was detected due to its dissolution during the heating. Flow-through cross polarized light imaging demonstrated that β-carotene was partly crystalline even at low concentrations. Stability data showed that β-carotene content was 90% and 77% with Gelucire and Geleol, respectively.
Conclusions: This study showed that Hyper-DSC and XRPD analyzes were in agreement and had a sensitivity limit of 5% API. Flow-through cross polarized light imaging was more efficient than classical polarized light microscopy to confirm the presence of drug crystals. This new method has the potential to become an indispensible tool to characterize different kinds of solid dispersions and amorphous systems.

Keywords: Analytical methods, sensitivity limit, cross polarized imaging.

References:
[1] Reitz C, Kleinebudde P. Eur J Pharm Biopharm 2007; 67: 440–448.
[2] Bikiaris D et al. Thermochim Acta 2005; 439: 58–67.

P-26

Abietane Diterpenoids from Roots of *Salvia leriifolia* - Stereochemical Characterization by Computational Electronic Circular Dichroism

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Introduction: *Salvia* species are particularly rich in diterpenoids, and they are responsible for various biological activities of these plants. They possess diverse biological properties, such as antiinflammatory, cytotoxic, antibiofilm, antimicrobial, and anticancer activities [1].
Aims: In a project directed to the search for secondary metabolites from endemic Iranian Lamiaceae we investigated an *n*-hexane extract from roots of *Salvia leriifolia*.
Methods: Phytochemical profiling of an *n*-hexane extract by a combination of normal phase column chromatography and preparative and semi-preparative reversed phase HPLC afforded 6 diterpenoids. Their structures were established by means of extensive NMR (1D and 2D) and HRESI-MS spectroscopy. Finally, the absolute configuration of isolated compounds was established by comparing experimental and calculated electronic circular dichroism (ECD).
Results and conclusion: Six diterpenoids were isolated from *S. leriifolia*. The compounds have been previously reported from other Lamiaceae [1] but their absolute configuration was not yet elucidated. We determined the absolute configuration by ECD [2]. Data were calculated using timedependent density function theory TDDFT/B3LYP/6-31G** in MeOH as solvent, and the *"selfconsistent reaction field"* method (SCRF) with the conductor-like polarizable calculation model (CPCM) were used for calculation. A good match of experimental ECD spectra with calculated data led to the absolute configuration as depicted in Fig. 1.

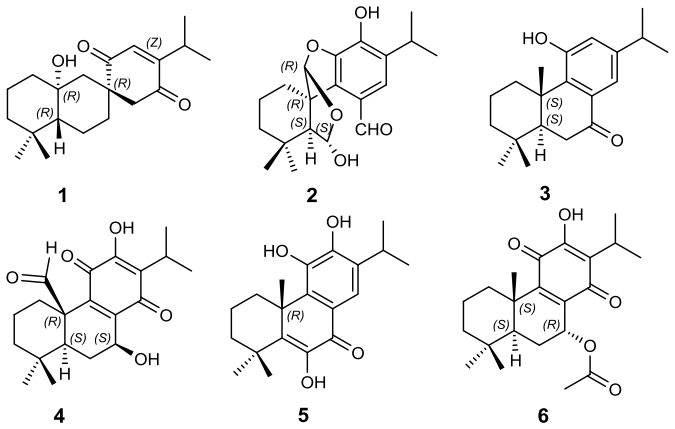

Figure 1 Diterpenoids of *Salvia leriifolia*

Keywords: Lamiacea, diterpenoids, *Salvia,* absolute configuration.

References:
[1] Araujo ECD, Lima MAS, Silveira ER. Magn Reson Chem 2004; 42: 1049-1052.
[2] Ebrahimi SN et al. Planta Med 2013; 79: 150-156.

P-27

Positively Charged Silica Nanoparticles Cause ROS in hCMEC/D3 Cells and Decrease Viability

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Introduction: Silica nanoparticles have attracted much attention in nanomedicine due to their vast versatility. Ease of surface functionalization allows the introduction of different chemical groups which deeply modify the main features of this nanomaterial. Silica nanoparticles are considered biocompatible, however, lack of knowledge regarding cytotoxic mechanisms hampers their application in clinics.
Aim and Methods: Cytotoxic effects of silica nanoparticles are herein investigated in hCMEC/D3 cells via MTT and ROS assays.
Results: Different silica nanoparticles have been synthesized bearing negative, positive and neutral surface charges. Interestingly, incubation of hCMEC/D3 cells with positively charged silica nanoparticles has shown a decrease in cell viability of up to 40% as well as a significant increase in production of cellular ROS.
Conclusion: A severe impact on hCMEC/D3 cell viability and cellular ROS production was observed upon incubation with charged silica nanoparticles. Whether these two facts are directly related is subject of investigation.

Keywords: Silica nanoparticles, drug delivery, reactive oxidative species (ROS).

P-28

PAMPA Technique Used to Predict Drug - Plasmatic Protein Binding Percentage in Two End-Point Measurement

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Introduction: A large number of high throughput screening (HTS) assays were developed to rapidly identify new chemicals entities (NCEs) with possible problems in ADMET phases (absorption, distribution, metabolism, elimination and toxicity). Parallel Artificial Membrane Permeability Assay (PAMPA) is a well-known HTS technique used to predict *in vivo* passive absorption. Two compartments are separated by an artificial membrane which mimic the passive permeability through biological membrane such as the dermal layer, gastrointestinal track (GIT) and the blood brain barrier (BBB) [1]. It was possible to use a modified PAMPA technique, to predict passive permeability and drug-protein interactions towards human serum albumin (HSA) [1]. HSA is with α-1-acid glycoprotein (AGP) the predominant proteins responsible for drug binding and distribution in the human plasma.
Aim: The aim of the present study was to elaborate a PAMPA strategy with the HTS hexadecane artificial membrane (HDM)-PAMPA in order to predict the percentage of binding affinity of a chemical compound towards the human plasma using mixtures of HSA and AGP in a two end-point measurement.
Methods: A first HDM-PAMPA without any protein in the acceptor compartment is performed to obtain passive permeability values. Those values are compared with a second PAMPA which contains the proteins in the acceptor compartment. The assay uses an equivalent physiological ratio of HSA and AGP found in the human plasma (~20:1) placed in the acceptor compartment respectively 100 µM and 5 µM.

Results: As obtained recently [1], the passive permeability measured in the conditions necessary to work with proteins (iso-pH 7.4) can discriminate compounds from no to high permeants. To increase the screening capacity, gradient pH HDM-PAMPAs (5.0 – 7.4) were performed as well to overcome the issue of passive permeability for acidic compounds. The percentage of binding affinity towards human plasma can be estimated via the correlation with the difference of compounds permeation between assays with and without proteins (AGP and HSA; $r^2 = 0.80$ obtained for 30 compounds). The modulation of drug permeation using HSA and AGP was compared to the one using separately HSA and AGP at the same concentration as in the protein mixture. A determination coefficient of 0.97 was obtained when comparing to HSA alone. No significant correlation was obtained comparing to AGP. However, despite the 20 times lower concentration used for AGP than HSA, a variation of compounds permeation with and without AGP was still observed. Furthermore a $r^2 = 0.85$ was obtained when comparing 16 compounds with their respective percentage of binding to AGP found in the literature. This demonstrates that AGP at used concentration can modulate the total plasma binding. Those observations suggest that the modified PAMPA-strategy can help to determine the percentage of binding towards HSA, AGP and human plasma.
Conclusions: The present study demonstrates the capacity of the PAMPA to determine the percentage of binding affinity towards plasmatic proteins by maintaining the prediction of passive permeability through the GIT. The study points out an open question on the influences which has AGP when performing an *in vitro* assay using an equivalent ratio of HSA and AGP as encounter in the human body.

Keywords: PAMPA, passive permeability, plasma protein binding, ADME.

Reference:
[1] Bujard A et al. Eur J Pharm Sci 2014; 63: 36-44.

P-29

Inhibition of Porcine Detrusor Contractility by a Flavonoid Fraction of Bryophyllum Pinnatum, a Potential Treatment for Overactive Bladder Syndrome

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Introduction: Patients with overactive bladder syndrome (OAB) suffer from urinary urgency, with or without urge incontinence, usually with frequency and nocturia [1]. Antimuscarinic agents are used as a first-line therapy with significant clinical benefits, but not seldom they exhibit undesirable anticholinergic side effects [2]. We recently investigated the inhibitory effect of the phytotherapeutic *Bryophyllum pinnatum* leaf press juice on porcine detrusor contractility *in vitro* [3].
Aims: To test the effects of *B. pinnatum* flavonoid, bufadienolide, and a highly polar fraction on the contractility of the porcine bladder strips in comparison to oxybutynin in order to determine if *B. pinnatum* could serve as an alternative drug for the OAB.
Methods: The methanolic extract of *B. pinnatum* was partitioned between CH_2Cl_2/H_2O to yield a fraction of lipophilic bufadienolides. The aqueous phase was separated on a Diaion HP-20 column in a flavonoid fraction, and a fraction of highly polar constituents. Detrusor muscle strips used for the contractility experiments were prepared from porcine bladders. The effect of the 3 fractions and oxybutynin on detrusor muscle contractility was investigated in an organ bath chamber. Muscle contractions were induced by electric field stimulation (EFS).
Results: The flavonoid fraction (1 mg/ml) showed a significant reduction of the contractility to 21.3 ± 5.2% after 77 min. The bufadienolide fraction had no inhibitory effect at the test concentrations. The polar fraction reduced the contractility in a pH-dependent manner, and oxybutynin (10^{-6} M) reduced the contractility to 21.9 ± 4.7% of the contractility measured before treatment (100%).
Conclusions: The flavonoid fraction reduced the porcine detrusor contractility in a dose- and time-dependent manner comparable to oxybutynin, a standard synthetic anticholinergic drug, in the treatment of OAB. *B. pinnatum* might be a new pharmacological option for the treatment of OAB.

Keywords: *Bryophyllum pinnatum,* flavonoids, bufadienolides, overactive bladder, detrusor muscle.

References:
[1] Haylen BT et al. Neurourol Urodyn 2010; 29: 4-20.
[2] Veenboer P, Bosch JL. J Urol 2014; 191: 1003-8.
[3] Schuler V et al. Phytomedicine 2012; 19: 947-51.

P-30

Analysis of Neopterin and Analogous Compounds by CE-MS

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Introduction: Pteridines belong to a broad family of compounds known for their implication in various biological systems such as the synthesis of tyrosine, serotonin, L-dopa, and nitrite oxide. Tetrahydro-biopterin (BH4) is a precursor of numerous pathways, including important inflammatory processes. Recently, neopterin (NEO) appeared as an efficient biomarker for human African trypanosomiasis and subarachnoid hemorrhage where its concentration was significantly increased in cerebrospinal fluid (CSF) and plasma, respectively. Because BH4, NEO and analogous mole-cules are highly polar (log P between -1.1 and -2.4) and ionizable, the on-line combination of capillary electrophoresis (CE) with mass spectrometry (MS) appears as an appropriate method for trace analysis of pteridines in biological fluids.
Methods: In this study, 11 compounds involved in the biosynthesis of BH4 and NEO were selected. The main objective of the study was to develop CE-MS compatible conditions for the simultaneous detection of all selected compounds. The CE method was hyphenated to ESI-MS/MS in SRM mode with a sheath liquid interface. It has to be noted that all analytes showed a significant stability issue: NEO is the oxidized form of dihydro-neopterin (NH2) while biopterin (BIO) is the oxidized end-form of dihydro- (BH2) and BH4. To ensure a sufficient stability of stock solutions, the latter have to

be prepared in an aprotic solvent (acetonitrile) and prior to dilution in water.

Results: Because pteridines are amphoteric compounds, two modes of separation were investi-gated, i.e. capillary zone electrophoresis (CZE) under acidic and basic conditions where a complete separation of the 11 molecules was obtained with a background electrolyte (BGE) made of 160 mM borate adjusted with ammonia to pH 10.3. This separation was achieved thanks to the specific interactions between borate and the vicinal diols of the pteridines. In order to enable MS hyphenation while maintaining effective interaction, the former BGE was then substituted by a volatile electrolyte (heptafluoro-isopropanol) mixed with 60 mM of borate adjusted to pH 10.3 with ammonia. Due to the low flow rates experienced in CE-MS, neither source contamination nor ESI signal suppression was observed. Another MS-compatible method was also investigated with an acidic BGE. Given their low basic pKa values, a highly concentrated formate BGE was used (formic acid 2%, i.e. 525 mM). All compounds were ionized and separated, except isoxanthopterin and guanosine triphosphate, which migrated as neutral and negatively charged compounds, respectively.

Conclusions: Two CE orthogonal separation methods, based on the amphoteric characteristic of pteridines, were developed. Firstly, a selective and sensitive (down to 1 ng/mL) separation was accomplished with an acidic BGE consisting of 2% formic acid. Under this condition, all compounds were separated except for GTP and the isomeric compounds neopterin / monapterin. Secondly, in order to achieve a total separation of all pteridines, an optimized buffer consisting of 62.5 mM hexafluoro-isopropanol and 60 mM boric acid pH 10.3 was used. No signal intensity loss was observed after 4 weeks of daily analysis, which corresponds to the recommended normal frequency for mass spectrometer cleaning. This method allowed a sensitive detection with a LOD down to 10 ng/mL.

Keywords: Biomarkers, pteridines, capillary electrophoresis, mass spectrometry.

P-31

Development and Optimization of an *In Vitro* Dissolution System for Inhaled Drug Products

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Introduction: *In vitro-in vivo* correlation *(IVIVC)* has been established between *in vitro* dissolution data and pharmacokinetic data for many systemically available products. *In vitro* dissolution testing of an inhaled drug has the potential to provide predictive estimates of its behavior *in vivo.* Such correlations enable the use of dissolution data for assessing post-approval changes to the formulation or manufacturing process, as well as for the development and approval of generic products. However, to date, no standardized methodology for the dissolution of orally inhaled products (OIPs) has been approved by regulatory authorities (US Food and Drug Administration, FDA) [1].

Aims: The aim of the FDA-sponsored project is to develop and validate a robust *in vitro* dissolution assay which may be used for *in vitro-in vivo* studies as well as for quality testing.

Methods: A custom made 25-mm modified Transwell® system was used for the dissolution tests (produced by a 3D-printer; material: polylactic acid) [2]. A glass microfiber filter was used acting as membrane between the donor and receptor compartment [3]. Particle fractions were collected from stage 4 of an Andersen Cascade Impactor (ACI) and a Next Generation Impactor (NGI). Fluticasone propionate (FP) and ciclesonide (CIC) were used for the experiments. To prevent a formation of agglomerates on the glass microfiber filter while drug collection in the ACI, a stage extension spacer was tested. The spacer extends the distance between the stages so that the powder may be distributed more homogenously.

Results: The dissolution profiles of the tested hydrophobic corticosteroids are greatly improved by using a medium containing 0.5% sodium dodecyl sulfate as a surfactant. By introducing a new glass microfiber filter, diffusion was no longer the rate limiting step. When the spacer was used, no significant effect on dissolution rate was visible so far. More amount of drug would be needed to see a change on dissolution. Dissolution times vary between the stages of an ACI. Stage 4 showed the fastest dissolution profiles for FP. Considering the different particle sizes that deposit on the different stages, these results seem comprehensible (larger particles get deposited on the upper stages and have a slower dissolution rate). According to the Noyes-Whitney equation, dissolution rates are related to the surface area of the dissolving substance exposed to the solvent.

Conclusions: The 3D-printed Transwell® system is well situated for dissolution testing of OIPs. Use of surfactant such as sodium dodecyl sulfate is essential for the *in vitro* dissolution of the tested hydrophobic drugs since under these conditions dissolution profiles are similar to absorption profiles observed *in vivo.* Replacing the polycarbonate membrane with a glass microfiber filter made dissolution the rate limiting step. Stage 4 of the ACI showed the fastest dissolution profiles for FP. Drug loading affects dissolution significantly.

Keywords: Inhaled corticosteroids, dissolution testing, cascade impactor studies, modified Transwell® system, *in vitro-in vivo* correlation.

References:

[1] Riley T et al. AAPS Pharm Sci Tech 2012; 13: 978–89.

[2] Arora D, Shah K, Halquist MS, Sakagami M. Pharm Res 2010; 27: 786–95.

[3] Rohrschneider M. Correlation of ICS in vitro dissolution and pulmonary absorption; Ph.D. Thesis. Heinrich-Heine Universität Düsseldorf: Germany; 2012.

P-32

Antitrypanosomal Activity of Quinoline Alkaloids from the Roots of Waltheria Indica

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Introduction: *Waltheria indica* L. (Malvaceae) is a short-lived shrub that can reach up to 2 m in height and is widespread in subtropical and tropical regions. Many ailments are treated with this plant such as skin ulcer, rheumatism, diarrhea, hemorrhoids, asthma and teeth

infection [1]. In Burkina Faso, the roots and aerial parts are used against malaria, whereas in Niger/Nigeria herdsmen give the whole plant to cattle as a tonic, suggesting a possible activity against animal sleeping sickness [2].
Aims: Extracts were prepared from the roots and the aerial parts and screened against protozoa from the genera *Trypanosoma, Leishmania* and *Plasmodium* with the aim to discover new natural products with antiparasitic activity.
Methods: A fractionation of the crude CH_2Cl_2 root extract was carried out by medium pressure liquid chromatography (MPLC). Structural elucidation was performed by NMR including ^{1}H-, ^{13}C-NMR, HSQC, HMBC, COSY and NOESY experiments, UV, IR, and mass spectrometry. The absolute configuration of compounds was established by comparison of experimental and TDDFT calculated ECD spectra.
Results: Twelve quinoline alkaloids were isolated and characterized, i.e. 8-deoxoantidesmone (Fig. 1: **1**), waltheriones A and C (**2, 3**), waltheriones E-K (**4-10**), 1-demethoxy-4-methoxy-waltherione G*N*-oxide (**11**) and antidesmone (**12**). To the best of our knowledge, compounds **4-11** have not yet been described in the literature. Amongst them, compounds **6, 7** and **10** showed potent and selective growth inhibition towards *Trypanosoma cruzi* with IC_{50} values between 0.02 and 0.04 µM, which is significantly lower than the reference drug benznidazole (2.22 µM). The alkaloids **1, 4, 6, 7, 9** and **10** showed significant toxicity towards mouse skeletal L-6 cells (IC_{50} values < 1 µM) whereas compound **3** exhibited low cytotoxicity (IC_{50} of 101 µM) when compared to its antitrypanosomal activity (IC_{50} of 1.93 µM), resulting in a selectivity index (IC_{50} L-6/IC_{50} *T. cruzi)* superior to 50. Hence this alkaloid conforms to hit activity criteria as required by the WHO/TDR with respect to *T. cruzi* [3].
Conclusion: Waltherione C (**3**) shows potent *in vitro* activity towards *T. cruzi* which encourages further investigations in order to evaluate the *in vivo* activity and to determine the mechanism of action.

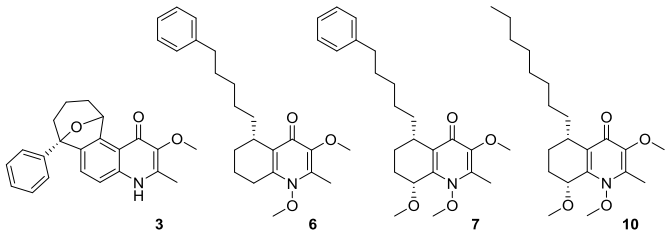

Figure 1 Structures of compounds 3, 6, 7 and 10

Keywords: *Waltheria indica,* quinoline alkaloids, antitrypanosomal activity.

References:
[1] Zongo F et al. J Ethnopharmacol 2013; 148: 14-26.
[2] Bala AY et al. J. Basic Appl. Sci. 2009; 17: 257-264.
[3] Nwaka S, Hudson A. Nat Rev Drug Discov 2006; 5: 941-955.

P-33

Misuse of Statistics in Experimental Pharmacology - An Evaluation of Six Journals

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Introduction: Biostatistics are inherent to life science. Their correct use minimizes the incidence of false positive or false negative results and ensures experimental reproducibility. Biologists misuse and misunderstand the fundamental concepts in biostatistics, hence a mounting concern about the proportion of irreproducible data.
Aims: In this study we quantified the articles that failed to comply with statistical guidelines with respect to design/analysis or display, in 6 pharmacology periodicals in 2014.
Methods: Six journals were included: *Neuropsychopharmacology, British Journal of Pharmacology, Biochemical Pharmacology, Neuropharmacology, BMC Pharmacology and Toxicology,* and *European Journal of Pharmacology.* Online archives were scanned and the 9-10 most recent articles were downloaded (April 1st 2014). Evaluated criteria (13 items) were scored 0 (item respected in all figures and tables) or 1 (flaw detected in at least 1 figure or table). Investigators were not blind to journal or authors identity.
Results: We found that inappropriate design or analytic procedures were frequent, including no reliance on bounds of 95% confidence intervals (i.e. sole reliance on p-value) to conclude (58/59 articles; 98%), underpowered or unknown sample size (43/59 articles; 73%), failure to respect the assumptions for parametric tests (49/57 articles; 86%), and uncorrected multiple comparisons (13/56 articles; 23%). Other frequent flaws in display included the use of standard error to show variability (39/54 articles; 72%), no exact p-values provided (49/57 articles; 86%), unknown error bars (12/59 articles; 20%), absence of statistical paragraph (1/59 articles; 2%). Compliance rate was not correlated with journal impact factor (R^2_{TOTAL} = 0.02; $R^2_{DESIGN/ANALYSIS}$ = 0.03; $R^2_{DISPLAY}$ = 0.00).
Conclusions: These results show that experimental pharmacologists misuse biostatistics and suggest a high occurrence of false positive and false negative results in publications.

Keywords: Statistics, design, journals, presentation, publication.

P-34

Production of Microparticles Containing Nanosized Drug Substance for Pulmonary Application

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Introduction: The interest in nanoparticles for pharmaceutical purposes as well as their importance in pulmonary drug delivery is rising [1]. By producing drug nanoparticles, for instance by wet media milling, the dissolution rate can be enhanced, which is especially valuable for poorly water-soluble drugs [2]. Spray drying enables embedding of nanoparticles into microparticles, which improves the nanoparticles' stability and application. A particle engineering approach to the spray drying process allows the production of particles with superior properties by better understanding of the particle formation mechanism [3].
Aim: Our aim was the preparation of engineered particles with improved aerosolisation properties and dissolution rate compared to unprocessed drug substance of approximately the same geometric diameter.
Methods: We produced a stabilised nanosuspension of budesonide by wet media milling. Subsequently, we spray-dried the nanosuspension using the particle engineering approach to tune the particles' aerodynamic properties and to increase the nanosuspension's stability. The particles were co-sprayed using additives such as mannitol, leucine, and glycine. The resulting powders were characterised in terms of geometric and aerodynamic particle size, morphology, and shape. Furthermore, the dissolution rate of aerodynamically classified particles was measured.

Results: The samples containing leucine showed the most favourable aerodynamic and dissolution behaviour. With leucine we achieved the highest fine particle fraction (72.7%), which was ~ 40% more compared to raw budesonide. Such formulation dissolved approximately 2-times faster than pure budesonide.
Conclusions: In this work, we present powders with enhanced aerodynamic properties and dissolution rate, and a high fine particle fraction compared to raw budesonide. The particle engineering proved as a valuable tool for production of nanoparticle-containing microparticles with improved properties relevant for pulmonary drug delivery.

Keywords: Wet media milling, nanoparticles, spray drying, leucine, fine particle fraction.

References:
[1] Sung JC, Pulliam BL, Edwards DA. Trends Biotechnol 2007;25: 563-570.
[2] Shegokar R, Müller RH. Int J Pharm 2010; 399: 129-139.
[3] Vehring R. Pharm Res 2008; 25: 999-1022.

P-35

Metabolite Profiling for Caffeic Acid Oligomers in *Satureja Biflora*

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Introduction: The genus *Satureja* (family of Lamiaceae) comprises approx. 30 species with worldwide distribution. Oils obtained from aerial parts of Satureja spp. are used as flavoring agents, herbal medicines and perfumes. The essential oil composition of different *Satureja* species has been thoroughly studied, but limited information is available on non-volatile secondary metabolites. *S. biflora,* known as "lemon savory", grows widely in different parts of Africa. The plant has been used in Kenyan and Tanzanian traditional medicine as antimicrobial, spasmolytic, diuretic, analgesic, and cicatrizing herb [1].
Aims: Aim of this study was a metabolite profiling of non-volatile secondary metabolites in the aerial part of *S. biflora.*
Methods: Separation of the methanolic extract by a combination of Diaion HP-20, Sephadex LH-20, and preparative and semi-preparative reversed phase HPLC afforded 9 compounds. Their structures were established by means of extensive 1D- and 2D-NMR, HRESI-MS, and electronic circular dichroism (ECD).
Results and Conclusions: One new and 8 known natural products were characterized, namely luteolin 7-O-β-D-glucuronide, rosmarinic acid, melitric acid A (**4**), methyl melitric acid A, clinopodic acid I (**6**), clinopodic acid K, clinopodic acid O (**8**) and clinopodic acid P (**9**) (Fig. 1). Compound (**1**) was a new flavone glycoside, and its absolute configuration was established by comparison of experimental and calculated ECD spectra. *S. biflora* is a rich source of caffeic acid oligomers.

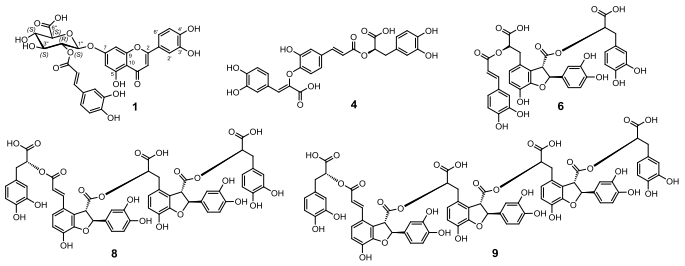

Figure 1 Selected compounds from *S. biflora*

Keywords: *Satureja,* lamiaceae, caffeic acid derivatives, flavonoids.

Reference:
[1] Matasyoh JC et al. Bull Chem Soc Ethiopia 2007; 21: 249-254.

P-36

Effect of Medetomidine on Ketamine N-Demethylation in Canine Liver Microsomes Analyzed by Enantioselective Capillary Electrophoresis

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Introduction: The analgesic and anesthetic drug ketamine is metabolized to norketamine as the main metabolite. This N-demethylation is catalyzed by cytochrome P450 (CYP) enzymes, which are located in the liver and other organs [1]. Dexmedetomidine, an α_2-adrenergic receptor agonist which effects sedation and analgesia, is the eutomer of medetomidine and a substrate for CYP enzymes [2]. Dexmedetomidine is used as comedication of racemic or S-ketamine in humans and dexmedetomidine or racemic medetomidine together with ketamine is used in animals, including canines and equines. Thus, drug drug interactions are likely to occur and it is important to characterize them.
Aims: The goals of this work were (i) to investigate the interaction *in vitro* between ketamine and medetomidine in canine liver microsomes related to the N-demethylation of ketamine to norketamine and (ii) to characterize the inhibitory effect of dexmedetomidine on this reaction with determining the inhibition parameters IC_{50} and K_i.
Methods: Canine liver microsomes were incubated at 37 °C for 8 min with racemic ketamine and separately with the single ketamine enantiomers using either 60 µM per ketamine enantiomer or, for K_i determination, 3 different substrate concentrations (0.5 Km, 1 Km and 2 Km). Medetomidine or dexmedetomidine were added in 5 concentrations between 0.075 and 0.9 µM. After incubation, the samples were extracted at alkaline pH with dichloromethane/ethylacetate (75:25 *v/v*) and reconstituted in 30 µL of 17.8 mM Tris-phosphate buffer pH 2.5. Samples were analyzed by enantioselective capillary electrophoresis using a running buffer composed of 17.8 mM Tris-phosphate buffer pH 2.5 and 2 % highly sulfated γ-cyclodextrin as the chiral selector. For determining IC_{50} and K_i the norketamine formation rate was plotted against the dexmedetomidine concentration and the data were analyzed as described previously [3].
Results: Both, racemic medetomidine and dexmedetomidine, show an inhibition of the N-demethylation of ketamine to norketamine, in which medetomidine is the stronger inhibitor. No stereoselective difference related to the substrate ketamine was observed. For dexmedetomidine, the data of the incubations with the different substrate and inhibitor concentrations could be fitted to the four-pa-

rameter-logistic model and provided IC_{50} values between 0.014 and 0.096 µM. The use of the Cheng-Prusoff equation revealed K_i values between 0.0074 and 0.054 µM.
Conclusions: Medetomidine and dexmedetomidine inhibit the N-demethylation of ketamine in presence of canine liver microsomes. Enantioselective capillary electrophoresis was successfully used for characterizing this inhibition. Further studies, especially *in vivo,* have to show the relevance and the consequences of this drug-drug interaction in clinical practice.

Keywords: Canine liver microsomes mediated N-demethylation, capillary electrophoresis, inhibition constant, ketamine, medetomidine.

References:
[1] Sinner B, Graf BM. Handb Exp Pharmacol 2008; 182: 313-333.
[2] Duhamel MC, Troncy É, Beaudry F. Biomed Chromatogr 2010; 24: 868-877.
[3] Kwan HY, Thormann W. Electrophoresis 2011; 32: 2738-2745.

P-37

Sunitinib- and Sorafenib-Eluting Biodegradable Microspheres for Transarterial Chemoembolization

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Introduction: Transarterial chemoembolization is a treatment option where embolic microspheres occlude the vessels feeding the tumor. In addition, the microspheres can be loaded with antiangiogenic agents to stop the tumor's survival response, which is to promote blood vessel formation and is called neoangiogenesis. The purpose is hence to reduce tumor size and ideally to prepare the patient for surgical resection, which offers highest survival chances. There is a clinical need for the microspheres to be biodegradable in order to limit post-operative risks, such as non-local embolization, and for the treatment to be repeated in case of tumor relapse.
Aims: To produce biodegradable microspheres ranging in size from 100 µm to 300 µm, composed of acrylic monomers and to evaluate the biodegradability, elasticity, loading and release of antiangiogenic drugs (sunitinib and sorafenib) from the microspheres.
Methods: Biodegradable microspheres were produced from acrylic monomers and PEG-diacrylate (m.wt.: 6000 g/mol) as the biodegradable cross-linker using an inverse suspension polymerisation. Biodegradability of the microspheres was evaluated over a period of 4 weeks at 37 °C with lateral shaking at 80 rpm in a phosphate buffer solution at pH 7.4. Elasticity of the microspheres was determined using oscillatory rheology with a plate-plate set-up, with strain and frequency parameters optimised for measurement purposes. Loading of sunitinib and sorafenib was performed for a nominal loading of 40 mg of drug/g of lyophilised microspheres and determined by spectroscopy at a wavelength of 430 and 265 nm, respectively. Release of sunitinib and sorafenib was done under sink conditions similar to those used for loading.
Results: Microspheres of an average size of 175 µm and a Young's modulus of 368 Pa were produced, showing good elasticity compared to commercial spheres. Biodegradation of the microspheres was observed to start after 2 weeks and to be ongoing at 4 weeks. Loading capacity of the microspheres for sunitinib and sorafenib was of 90% and 22%, respectively. Release of sunitinib and sorafenib reached a plateau of 40% and 10% after 3 h, respectively.
Conclusions: Biodegradable microspheres within the targeted size range were produced successfully. The spheres were highly elastic, which allows for an easy passage through a catheter during embolisation procedures. High loading of sunitinib was achieved, while sorafenib loading was lower, as the formulation of the stock solution still requires optimisation. Release for sunitinib was sufficient to yield therapeutic concentrations.

Keywords: Acrylic microspheres, chemoembolization, sunitinib, sorafenib, biodegradation.

P-38

In Silico Structural and Thermodynamics Studies of Protein-Protein Interactions Involving Ubiquitin and HDAC6 ZnF-UBP Domain

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Introduction: HDAC6 is a class II histone deacetylase (HDAC) able not only to catalyze the removal of acetyl groups from α-tubulin and from other cytoplasmic substrates but also to interact with free unanchored mono- and polyubiquitin chains through a zinc-finger ubiquitin (Ub) binding domain (ZnF-UBP). HDAC6-Ub interactions are related with the degradation of misfolded protein aggregates through the aggresome pathway, which is activated when the proteasome is impaired. The ZnF-UBP domain of HDAC6 [1] is structurally related to the one described for USP5 [2], a deubiquitinating enzyme involved in the disassembly of unanchored polyubiquitin chains released after proteasomal degradation of target proteins.
Aims: This study aimed at understanding the dynamics and thermodynamics of Ub recognition by HDAC6 ZnF-UBP domain by using a molecular dynamics (MD) approach. USP5 ZnF-UBP, the prototypical ZnF-UBP domain, was also analyzed at atomic level and compared to HDAC6 ZnF-UBP.
Methods: Structural and solvation features of Ub in complex with HDAC6 and USP5 ZnF-UBP domains were analyzed by MD simulation over 120 ns. Theoretical free energies of binding were determined by using the MM-GBSA approach. Moreover, a computational alanine scanning was performed to estimate the individual energy contribution of residues essential for the binding. The results were compared with experimental data obtained in the past by crystallography and by isothermal titration microcalorimetry (ITC) [2,3].
Results: ZnF-UBP and Ub interactions were studied by MD focusing on HDAC6 and USP5 systems. New structural and thermodynamics information were retrieved completing the structural and energetic scenario given by crystallography and ITC data [1-3]. Despite the overall stability characterizing the 2 systems, MD highlighted zones of structural diversity, not detected by crystallography. The analysis of the solvation network at the protein-protein binding interface revealed the importance of 2 water molecules to stabilize the complexes formed between Ub and the ZnF-UBP domains of HDAC6 and USP5. The theoretical free energies of binding were in line with the experimental binding affinities and the computational alanine scanning revealed the hotspot residues for Ub binding in ZnF-UBP domains.
Conclusions: New mechanistic insights in Ub recognition by ZnF-UBP systems were provided. Flexibility, solvation and thermodynamics features were described here for the first time. The exclusive structural features highlighted for HDAC6 ZnF-UBP – Ub recognition, indicated the potential hotspots for the rational design of compounds aiming at investigating HDAC6-mediated cellular responses.

Keywords: Histone deacetylase 6, molecular dynamics, protein-protein interaction, ubiquitin, ZnF-UBP domains.

References:
[1] Ouyang H et al. J Biol Chem 2012; 287: 2317-2327.
[2] Reyes-Turcu FE et al. Cell 2006; 124: 1197-1208.
[3] Boyault C et al. Embo J 2006; 25: 3357-3366.

P-39

Vincristine-loaded Liposomes for Targeted Rhabdomyosarcoma Delivery

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Introduction: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and displays a highly aggressive and metastatic behavior. Although current treatment regimens, including surgery and chemotherapy, can achieve good response rates, late side effects represent a heavy burden for cancer survivors. A peptide with strong affinity for RMS *in vitro* and *in vivo* has been identified to selectively deliver drugs to the tumor. Experiments with doxorubicin coupled to the targeting peptide in tumor-bearing mice resulted in a higher therapeutic efficacy compared to the chemotherapeutic drug alone, as proved by a 2-fold delay in tumor growth [1].
Aims: The aim of the present work is to develop a long-circulating anticancer liposomal formulation, decorated with the RMS-specific peptide. Such vesicles will selectively recognize furin, a proprotein convertase, overexpressed on the surface of RMS.
Methods: The targeted liposomes were prepared by incorporating 5 mol% of a NHS ester of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[carboxyPEG-2000] (DSPE-PEG-NHS) chemically coupled to the selected peptide. The best coupling conditions were found to take place in DMSO with a 5 times excess of NHS *vs.* RMS-peptide. Vincristine (VCR), one of the drugs for first line RMS treatment, was used in this study as therapeutic agent and was encapsulated into 130-nm liposomes following a transmembrane pH-gradient procedure.
Results and Conclusions: Encapsulation efficiency of 90% was achieved for both, targeted and not labeled liposomes and a release of 20% of the entrapped drug from the peptide-decorated vesicles in the presence of 100% serum within 24 h demonstrated that these liposomes slowly delivered VCR. The selective binding of the RMS-specific liposomes will then be tested *in vitro* in RMS cell lines overexpressing furin and later on *in vivo* in tumor-bearing mice.

Keywords: Rhabdomyosarcoma, tumor cell targeting, PEG-coated liposome, furin, vincristine.

Reference:
[1] Hajdin K et al. PLoS One 2010; 5: e10445.

P-40

Case-control Analysis on Metformin and Cancer of the Esophagus

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Introduction: Metformin use has been associated with decreased cancer risks, though data on esophageal cancer are scarce.
Aims: To explore the relation between use of metformin or other anti-diabetic drugs and the risk of esophageal cancer.
Methods: *Design:* Case-control analysis. Cases were individuals with an incident diagnosis of esophageal cancer between 1994 and 2010 at age 40-89 years. Ten controls per case were matched on age, gender, calendar time, general practice, and number of years of active history in the UK-based General Practice Research Database (now Clinical Practice Research Datalink, GPRD) prior to the index date. In the main analysis, the index date was shifted back by 2 years in time both for cases and controls (i.e., we assessed all exposure and covariate information 2 years immediately prior to the recorded index date), to take into account the latency of the disease diagnosis. Various potential confounders including diabetes mellitus, gastro-esophageal reflux, and use of proton pump inhibitors were evaluated in univariate models, and the final results were adjusted for BMI and smoking. *Setting:* CPRD. *Exposure:* Number of prescriptions of anti-diabetics drugs before the index date. *Main outcome measures:* Results are presented as odds ratios (ORs) with 95% confidence intervals (CI). *Statistical analysis:* Conditional logistic regression.
Results: Long-term use (≥ 30 prescriptions) of metformin was not associated with a materially altered risk of esophageal cancer (adj. OR 1.23, 95% CI 0.92-1.65), nor was long-term use of sulfonylureas (adj. OR 0.93, 95% CI 0.70-1.23), insulin (adj. OR 0.87, 95% CI 0.60-1.25) or thiazolidinediones (adj. OR 0.71, 95% CI 0.37-1.36).
Conclusion: In our population-based study, the use of metformin was not associated with an altered risk of esophageal cancer.

Keywords: Metformin, cancer, case-control study.

P-41

Identification of the α-Subunit of AMP-Activated Protein Kinase and Characterization of the Entire Complex in *Trypanosoma brucei*

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Introduction: AMP-activated protein kinase (AMPK) is a heterotrimeric complex well conserved in all eukaryotes, which acts as a regulatory sensor of the energy status of the cells. Its role is based mostly on sensing the AMP/ATP ratio of the cells and to restore the adenine nucleotide homeostasis [1]. In the blood stream form of *Trypanosoma brucei,* the parasite responsible for the Human

African Trypanosomiasis or sleeping sickness, the AMPK complex has neither been completely identified nor characterized to date. Previous investigations on the procyclic form of *T. brucei* allowed the identification of the β and γ subunits of TbAMPK. Two possible α subunit candidates have been identified by sequence homology, but further tests using RNAi methods failed to confirm the role of one of the two candidates in procyclic form of *T. brucei.*

Aims: It is the aim of this project to confirm the identity of the β- and γ-subunits and to identify the unknown α- subunit in the blood stream form of *T. brucei.*

Methods: To achieve this, two main methologies are being employed. (1) Polycistronic prokaryotic co-expression of different TbAMPK subunits/isoforms in order to identify at least one α- candidate, and (2) the use of the proximity-dependent biotin identification (BioID) assay to immunoprecipitate all biotinylated proteins that are in a complex with the β-subunit in *T. brucei.*

Results: We hare generating two polycistronic expression systems containing the β-, γ- and the 2 putative α-subunits, respectively. We will express the construct in *E. Coli* and purify the soluble and functional fusion protein thanks to the His-tagged α- subunit. Previous work demonstrated the capacity of this approach to isolate active recombinant AMPK complexes in mammalian [3].

In parallel, and as numerous trials using standard immunoprecipitation protocols failed to provide a suitable candidate for the α-subunit, we are also applying the recently developed BioID assay. This system consists of tagging one subunit with a modified 35-kDa bacterial biotin ligase (BirA*) harboring a myc-tag [2] and allow the identification of all elements building up the AMPK complex either by affinity purification via the myc-tag, or by immunoprecipitation of all biotin-labelled proteins after incubation of cells with biotin. In our case, we have successfully tagged the β-subunit in C-terminus with the BioID system and verification of the presence of the tagged protein in the transformed cells is now in progress.

Conclusions: Given the key role of the TbAMPK complex in the metabolism of *T. brucei,* it is very important to understand the components of this complex. With this work, we will shed light on the identity of the α- subunit of AMPK in *T. brucei,* thus providing valuable information on a potentially new therapeutic target for Human African Trypanosomiasis.

Keywords: *Trypanosoma brucei,* AMP-activated protein kinase, BioID.

References:

[1] Carling D. TIBS. 2004; 29: 18.
[2] Morriswood B et al. Eukariot Cell 2013; 12: 356.
[3] Neumann D et al. Protein Expr Purif 2003; 30: 230.

P-42

Anti-Proliferative Effect of Heterologous Expressed Nuclear Receptor NR0B2 in Renal Cell Carcinoma Cells

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Introduction: Mammalian nuclear receptors are transcription factors regulating expression of target genes which play an important role in drug metabolism, transport and cellular signaling pathways. One unique member of this protein superfamily is the orphan nuclear receptor *small heterodimer partner 1 SHP1* (NR0B2) which lacks the typical DNA binding domain of this transcription factor family. Current findings show that NR0B2 is down-regulated in human hepatocellular carcinoma and suggest that NR0B2 functions as a tumor via inhibition of cellular growth and activation of apoptosis [1].

Aims: The aim of our study was to test whether NR0B2 also plays an important role in other tumor entities such as renal cell carcinoma.

Methods and Results: Performing quantitative real-time RTPCR and Western Blot analysis revealed that NR0B2 expression was significantly lower in tumor samples of human renal cell carcinoma comparing non-malignant transformed tissue suggesting that mechanisms of reduced NR0B2 expression might also play a role in this tumor entity. To find a strategy to modulate NR0B2 expression in human kidney cells we performed adenoviral-transfer of NR0B2 in a renal cell carcinoma cell line (RCC-EW). To validate whether adenovirus-mediated transfer of NR0B2 was successful NR0B2 protein expression analysis of infected cells was conducted. Western Blot analysis showed that increasing amount of adenoviral load induced an increasing expression of NR0B2. Additionally, immunofluorescence staining of RCC-EW cells detected the abundance of NR0B2 after infection with NR0B2-inducing adenovirus (pAD-CMV-NR0B2).

After validation of adenoviral-transfer of NR0B2 the impact of heterologous expression of NR0B2 on cell cycle progression and proliferation was analyzed. Cell viability assays were performed by monitoring fluorescence intensity of resazurin turnover showing no significant differences in metabolic activity after viral transfer of NR0B2. However, there was a significant decrease of cellular proliferation in cells overexpressing NR0B2. Flow cytometry analysis showed that heterologous overexpression of NR0B2 significantly reduced the amount of cells passing G1 phase while on the other hand more cells were detected performing S/G2 phase.

Conclusions: Taken together, our findings reveal that NR0B2 is down-regulated in renal cell carcinoma. Our data also show that heterologous NR0B2 expression diminishes cellular proliferation of kidney tumor cells *in vitro* supporting its regulatory role in renal cell cancer progression. Future studies have to specify the mechanism responsible for diminished proliferation of renal carcinoma cells and the shift of cell cycle phases after overexpression of NR0B2.

Keywords: NR0B2, nuclear receptor, renal cell carcinoma.

Reference:

[1] Park YY, Choi HS, Lee JS. Mol Cells 2010; 30: 485-91.

P-43

Bioactive Scaffolds Releasing Engineered GDNF for Neural Tissue Regeneration

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Introduction: Peripheral nerve injuries affect more than one million people each year and often result in life-long disabilities. Development of bioactive scaffolds with topographical guidance and sustained neurotropic factors (NTFs) support may address some of the limitations associated with currently available treatment modalities, i.e., nerve grafting and artificial nerve conduits (NCs) [1].

Aim: This study aimed at investigating 2 approaches to control the release of glial cell line-derived neurotrophic factor (GDNF). For this,

we have engineered GDNF with collagen binding domain (CBD-GDNF) which was released from 2 delivery systems i.e., collagen NC scaffolds and poly lactic-co-glycolic acid (PLGA) microfibrous scaffold, respectively. Later approach was envisaged to integrate the PLGA guidance fibers into collagen NC.
Methods: Recombinant CBD-GDNF was cloned into eukaryotic vector pCDNA3, expressed in mammalian 911cell line and purified by immobilized metal affinity chromatography. Recombinant CBD-GDNF or native GDNF (50 ng/NC) was loaded into collagen NCs fabricated by spinning mandrel technology. For the other approach, PLGA solution was premixed with these growth factors (5 ng/mg) and fabricated into aligned microfibrous scaffold. Release kinetics were studied *in vitro* over 28 days from both collagen NC and PLGA scaffolds. Bioactivity of released NTFs was assessed by using Neuro-2A cell line. Furthermore, the ability of bioactive PLGA microfibrous scaffolds to promote axonal outgrowth was tested *in vitro* using chicken embryonic dorsal root ganglionic (DRGs) explant cultures [2].
Results: Collagen NC scaffolds showed sustained release for both GDNF and CBD-GDNF over 28 days. Interestingly, CBD-GDNF mediated slow and low release with significantly reduced initial burst release when compared to native GDNF. Another approach using PLGA scaffold also resulted in sustained release of growth factors, but the release profiles were similar for both GDNF and CBD-GDNF. The release rates were nearly constant over 28 days with only few picograms of daily release. Bioactivity of released NTFs was maintained throughout the entire release period as demonstrated by neuronal differentiation of Neuro-2A cells treated with NTFs release. Topography of the microstructed PLGA scaffold releasing NTFs determined the direction and extent of axonal outgrowth from DRG explants. Axonal outgrowth from DRGs was perfectly (99%) in line with the aligned fibers, but randomly oriented on non-aligned fibers.
Conclusions: Immobilization of NTFs in collagen NCs by fusion with collagen-binding domain is an appealing strategy for controlling release kinetics with reduced burst release and for sustaining bioactivity of NTFs. Alternatively, electrospun PLGA microfibers appeared to be appropriate delivery system for sustaining NTFs' slow release and holds potential interest for guiding the peripheral axonal regeneration. Future studies will assess the beneficial effects of collagen NC integrated with GDNF-PLGA microfibers in rat nerve gap model.

Keywords: Nerve regeneration, drug delivery, bioactive scaffolds, microfibers.

References:
[1] Madduri S, Michael P, Gander B. Biomaterials 2010; 31: 2323-34.
[2] Stoeckli E et al. J Cell Biol 1991; 112: 449-55.

P-44

Layer-by-Layer (LbL) Coated Biodegradable Microspheres for Chemoembolization of Liver Tumors

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Introduction: Drug-eluting beads (DEBs) for transarterial chemoembolization (TACE), an established clinical procedure for treatment of hypervascular tumors, comprises loading of pre-calibrated beads with anticancer or anti-angiogenic agents, followed by local injection to the tumor feeding arteries. This procedure allows to retain the injected chemotherapeutic agent close to tumour tissues to avoid systemic toxicity. However, available marketed DEBs still face several challenges. First, biodegradable beads are needed to allow multiple interventions on patients. Second, a more sustained drug release profile would be beneficial, for instance to deliver sunitinib, a multiple receptor tyrosine kinase inhibitor, to provide a long-lasting anti-angiogenic effect.
Aims: To develop, synthesize and fully characterize biodegradable microspheres for chemoembo-lization, load them with sunitinb and investigate the feasibility of Layer-by-layer (Lbl) coating to control drug release and suppress initial burst release.
Methods: Biodegradable microspheres were prepared using an inverse suspension polymerization with a biodegradable crosslinker. An aqueous phase containing the polymeric mixture (poly(ethylene glycol) diacrylate, acrylic acid, hydroxy ethyl methacrylate) was dispersed in cyclohexane through mechanical agitation. Size, zeta potential, elasticity and biodegradability were measured and morphology was investigated using scanning electron microscopy. The prepared microspheres were loaded with sunitinib by immersion in drug solution, followed by coating with alternating layers of alginate and poly-L-lysine as biocompatible polyelectrolytes. A series of formulations with increasing coating thickness were prepared and drug loading and release were quantified by spectrophotometry.
Results: Biodegradable microspheres were successfully synthesized with a yield higher than 80% and with the desired properties of size (184.81 ± 2.4 µm), zeta potential (-25.5 ± 0.5 mV) and elasticity (3.7±1.3 kPa). A change in pH, size, elasticity and shape could be clearly observed within 28 days, suggesting a biodegradation process. Microspheres were efficiently loaded with sunitinib reaching up to a loading capacity of 35.8 mg/100 mg dry spheres. Successful LbL coating was followed by measuring the surface potential of the particles which was inversed after the deposition of each successive layer. *In vitro* release profiles showed an inverse relation between LbL coat thickness and release rate and extent, where thick coats eventually led to a slow release profile over several days with a minimal burst release.
Conclusions: Novel biodegradable microspheres for anti-cancer chemoembolization have been developed and thoroughly characterized *in vitro.* Efficient loading and controlled release profiles may make this formulation promising for further development and *in vivo* experiments.

Keywords: Chemoembolization, layer-by-layer(LbL), biodegradable microspheres, sunitinib.

P-45

Investigation on Anthranoid Aglycones in Frangulae Cortex and Sennae Folium/Fructus with HPLC

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Introduction: Frangulae cortex, Sennae folium and Sennae fructus are used as laxative drugs in phytotherapy. Four monographs exist in the European Pharmacopeia for these 3 herbal drugs. In none of the monographs a method for the detection of the potentially mutagenic anthranoid aglycones (aloeemodin, chrysophanol, emodin, physcion, rhein) is mentioned. During the process change of old photometric assays to modern HPLC methods, the simultaneous detection of anthranoid glycosides and anthranoid aglycones swept back in the centre of attention.
Aims: An optimal HPLC-DAD method was sought for the detection of the 5 aglycones. A simultaneous detection together with the anthranoid glycosides would be preferred and if possible it should work for Frangulae cortex, Sennae folium and Sennae fructus. Several published methods have been evaluated [1].

Methods: The method of Rosenthal et al. [2] was the most powerful for the analyses of the 5 anthranoid aglycones. The original gradient had to be adapted and prolonged to separate the late eluting aglycones. The gradient step 76% ACN/MeOH up to 100% ACN/MeOH was extended from 26 to 36 min. The sample preparation remained unchanged, experiences with methanol instead of the mixture of 2% aqueous NaHCO3/ACN (320/680 *v/v*) – validated for the analyses of the glycosides - showed only a slight increase in the results.

Results and conclusion: Emodin, chrysophanol and physcion could be detected in Frangulae cortex Ph.Eur. and in herbal extracts thereof. Emodin is the main anthranoid aglycone (Fig.1). Anthranoid glycosides can be detected and quantified simultaneously with anthranoid aglycones in Frangulae cortex. The concentration of the anthranoid glycosides in Senna (aloeemodin and rhein) is lower. Therefore, it is probably not possible to analyse them together with the sennosides. Upper limits for the content of anthranoid aglycones in herbal drugs and herbal drug preparations may be determined after the analyses of even more samples (work in progress).

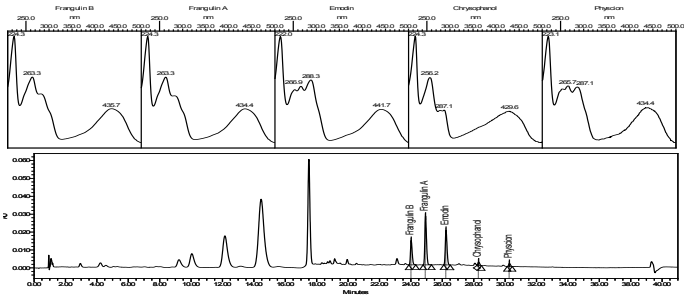

Figure 1 HPLC-DAD profile of anthranoid aglycones and glycosides in Frangulae cortex

Keywords: HPLC-DAD, Frangulae cortex, Sennae folium/fructus, anthranoid aglycones.

References:

[1] Weber N. Untersuchung zu Anthranodaglyka in Frangulae cortex und Sennae folium mit Hilfe verschiedener HPLC-Verfahren. Bachelor Theses. ZHAW Wädenswil, 2014.
[2] Rosenthal I et al. J Nat Prod 2014; 77: 489-496.

P-46

Use of a Design of Experiment Approach to Optimize the Fractionation of Subvisible Proteinaceous Particles by Differential Centrifugation

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Introduction: It has been controversially discussed whether proteinaceous particles potentially contribute to immunogenicity in biotherapeutics [1,2]. However, experimental assessment of the clinical relevance of such concerns is very challenging. Although a number of studies have been published, it is difficult to draw sound conclusions from the reported results. This is mainly due to the fact that in most cases complex mixtures of size-species (e.g. monomer, oligomers, and sub-visible particles) were tested, that also included a variety of different chemical species. Thus, even if any results were generated, it was unclear which aggregate size or chemical species would be responsible.

Aims: In order to overcome this drawback and obtain concentrated, well-defined, highly enriched size-fractions of sub-visible particles, a fractionation process for subvisible particles was deve-loped, based on a differential centrifugation approach.

Methods: Firstly, the basic experimental parameters to isolate 4 defined fractions of different sizes ranging from 0.2 µm to 100 µm were obtained by trial and error runs according to empirical Stokes law. In a second step, a design of experiment (DOE) approach was used for optimization. Response surface methodology (RSM) based on a central composite face centered design (CCFD) was employed to optimize the experimental parameters needed for the generation of each fraction.

Results and Conclusions: Using this procedure, 4 different highly concentrated and greatly enriched size-fractions were isolated from an artificially stressed IgG solution. In the future this methodology will be used to generate well-defined size-fractions of subvisible particles in order to enable characterization and *in vivo* studies of the properties of these subvisible particle species.

Keywords: Subvisible particles, fractionation, design of experiment, differential centrifugation.

References:

[1] Carpenter JF et al. J Pharm Sci 2009; 98: 1201-1205.
[2] Singh, SK et al. J Pharm Sci 2010; 99: 3302-3321.

P-47

Pharmacokinetics of Fibroblast Growth Factor-2

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Introduction: Fibroblast growth factor-2 (FGF-2) is an important cytokine localised in the extra-cellular matrix (ECM) that is involved in wound healing and embryogenesis. FGF-2 is found in an inactive form bound to different macromolecules (e.g. heparan sulphate) and has to be released to bind to FGF receptor tyrosine kinases expressed on cell membranes to induce biological activities. FGF-2 is known to activate FGF receptors on endothelial cells to stimulate angiogenesis (e.g. in tumours). Due to this angiogenic property, FGF-2 is already utilised in the clinic to treat e.g. cardio-vascular disorders, although the regulation of the FGF-2 signalling in the ECM is still poorly understood.

Aims: The aim of this project is to investigate the influence of different binding partners such as heparin sulphate on the distribution of exogenously administered FGF-2 *in vivo* by non-invasive small animal imaging (SPECT/CT). For this purpose a protocol for the radiolabelling of FGF-2 should be developed, avoiding the alteration

of the biological properties of the protein. The notorious instability of the protein makes this project particularly challenging [1].
Methods: FGF-2 was selectively radiolabelled at cysteine residues with $^{nat/111}$In using malemide DTPA as the chelator, leading to labelling yields higher than 98% (specific activity 5 MBq/nmol). The affinity of ^{111}In-DPTA-FGF-2 to the receptor was estimated by saturation binding assay on fibroblast cells, while isothermal titration calorimetry was used to determine the binding to low-molecular weight heparin. Finally, a first SPECT/CT analysis was performed with the radiolabelled FGF-2 in healthy CD1 nu/nu mice.
Results: For $^{nat/111}$In-DTPA-FGF-2 a K_d of 22 ± 4 nM was obtained from the binding assay, which is in accordance with published data of 60 nM for the native protein [2]. Both natIn-DTPA-FGF-2 and FGF-2 showed a similar affinity to low-molecular weight heparin (K_d: 0.6 ± 0.07 µM and 0.33 ± 0.03 µM). SPECT/CT pictures obtained 4 h p.i. showed a predominant accumulation of ^{111}In-DTPA-FGF-2 in the liver and the kidneys. Activity was also detected in the retina, salivary glands and the pituitary gland, which are known to express specific receptors for FGF-2 [3]. However, it is still unclear if the uptake is specific.
Conclusions: FGF-2 was successfully labelled with ^{111}In. *In vitro* experiments indicated that the binding to the receptor and to heparin was not altered by the labelling, while the first *in vivo* results suggest that the tracer has similar properties to the native protein. Since, at the moment we don't have information about the specificity of the accumulation *in vivo.* Further biodistribution study will be performed to investigate the specificity of the *in vivo* uptake.

Keywords: FGF-2, angiogenesis, SPECT/CT, ECM.

References:
[1] Estape D, Heuvel J, Rinas U. Biochem J 1998; 349: 343-349.
[2] Ibrahimi OA et al. J. Biochemistry 2004; 43: 4724-4730.
[3] Valter K et al. Growth Factors (Chur, Switzerland) 2002; 20: 177-188.

P-48

Evaluation of Subvisible Particle Counting Methods

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Introduction: Subvisible particles (SbVP) are ubiquitously present in protein formulations. The pharmaceutical community agrees in the necessity of monitoring them because of the potential - and yet not clear - risk regarding immunogenicity. There are different instruments for particle characterization commercially available. Therefore, it is very important to have detailed under-standing of the instruments' performance when analysing SbVP.
Aims: The goal was to explore method performance at relevant conditions for biotech products (particle load and morphology) using particle- and instrument-specific evaluation studies. This study evaluates various particle instruments (Nano Tracking Analysis, NTA; Coulter Counter, CC; Microflow Imaging, MFI; FlowCAM, FC and the compendial light obscuration method) in terms of counting accuracy. Conventional latex beads standards and 3 different models of actual protein particles produced under artificial heat-stress conditions were used.
Methods: Depending on instrument's specifications, dilutions ranging from 10^1 to 10^8 particles per mL were prepared by a well-defined and controlled dilution process. Experimental particle concentration was determined and results were processed by calculation of recovery percentage of expected *vs.* experimental particle counts. Finally, operator analysis by assessment on plotted results was applied to define lower limits of counting capabilities.
Results: The counting accuracy by recovery of all protein aggregates varied depending on the instrument's principle. Furthermore, this study suggested a strong relation between particle type and instrument performance. Below $1x10^3$ particles/mL, data showed that various techniques were in fact over-counting SbVP.
Conclusions: We have described the recovery profile of counting instruments in a particle- and instrument-specific manner. Our study has potential applicability towards further assessing of a meaningful array of particle counting techniques and defining the analytical performance of these methods including their limitations and the sources of error.

Keywords: Sub visible particles, particle counting instruments, protein aggregates.

P-49

Fogging in Lyophilized Drug Products

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Introduction: Vial fogging is a phenomenon observed after lyophilization due to drug product solution creeping upwards along the inner vial surface, being a cosmetic product defect. The main factor to control fogging is primarily the inner vial surface hydrophilicity/hydrophobicity. The usage of vials with a hydrophobic surface offers a solution for fogging [1]. However, surface hydrophobicity may also possibly be modified due to processing, such as the lyophilization process itself.
Aims: The purpose of this work was to investigate the influence of the lyophilization process parameters on the extent of fogging.
Methods: A protein formulation containing a monoclonal antibody (mAb) was freeze-dried in both hydrophilic vials (untreated) and hydrophobic vials (hydrophobic coating). Vials were washed and depyrogenized in-house prior to filling and freeze-drying. In addition, a fluorescein dye test was performed to verify the fogging effect on the raw material.
Results: Lyophilization process parameters had a minor impact on the extent of fogging. Nevertheless, fogging could not be eliminated by optimizing lyophilization process parameters. The usage of hydrophobic vials, however, showed reproducible results resulting in no fogging at all.
Conclusions: Fogging can be avoided when using vials with a hydrophobic coating, where the variations of the lyophilization process play a minor role.

Keywords: Fogging, lyophilization, hydrophobic.

Reference:
[1] U.S. Patent No 20120018338 (issued Mar 26, 2010).

P-50

New Approaches for Functional Genome Analysis in a Genetically Intractable System Organism *(Waddlia chondrophila)*

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Introduction: The incidence of Chlamydia infections in the world's population is still increasing and the lack of knowledge about this pathogen warrants novel interdisciplinary and broadly applicable pharmacogenetic strategies. Therefore, a functional study in a genetically intractable organism such as *Waddlia chondrophila,* a strict intracellular bacterial pathogen from the phylum *Chlamydiae,* was initiated. This bacterium possesses a particular developmental cycle starting from an infectious cell type (Elementary Body, EB) to a replicative one (Reticulate Body, RB) and back (Fig. 1).

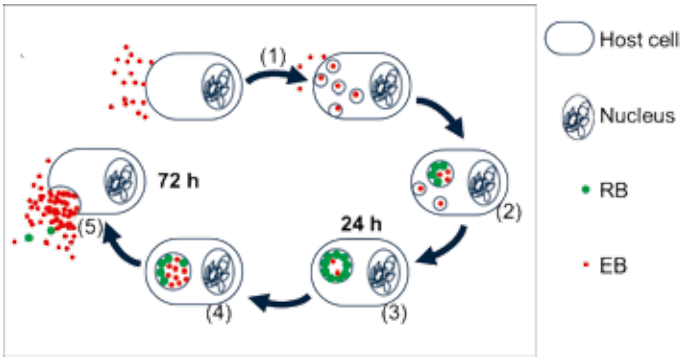

Figure 1 The Chlamydia developmental cycle where EBs infect the host cell (1), morph into RBs (2; EB→RB) that then proliferate (3). The RBs differentiate into EBs (4; RB→EB) that lyse the host (5)

Aims: In this project we aim (i) to identify molecules that alter the ability of *W. chondrophila* transcription factors (TF) to interact with DNA and (ii) to identify metabolites that are expressed differently during the developmental cycle of *W. chondrophila* from RB to EB and/or from EB to RB.

Methods: A core set of 10 TFs which are conserved in other species of the phylum *Chlamydiae* and likely involved in their development was chosen. A screening method was set up using a reporter assay in a heterologous system *(E. coli).* Each TF was separately cloned in a plasmid and its DNA-binding motif with a reporter system in another. The reporter systems *lacZ,* biolumines-cence (LuxAB) and kanamycin resistance were tested and the most suitable will be used for the final screening in a 96 well-plate. In parallel, a second approach using metabolomic studies is being performed on cell extracts. Mammalian cells infected with *W. chondrophila* were extracted 24 and 72 h after cell infection and analysed by UPLC-TOF/MS.

Results: The 3 reporter plasmids were cloned and expressed in *E. coli.* The reporter system containing kanamycin resistance can be used in a qualitative screening whereas reporter systems containing *lacZ* or luminescence reporters can be used for kinetic studies and quantification. In the metabolomic study, the set-up of the extraction method allowed the detection of chemical differen-ces between extracts from infected and non-infected cells. This data validate the extraction procedure and analysis.

Conclusions: The *lacZ* reporter system can be used as a screening system in 96 well plates. The promising results obtained in the metabolomic part will lead to scale-up experiments for further investigation and determination of chemicals of interest.

Keywords: *Chlamydia, Waddlia chondrophila,* transcription factors, metabolomics.

P-51

Needle-Free Cutaneous Deposition of Drug Loaded PLGA Microparticles Using P.L.E.A.S.E.® Fractional Laser Ablation

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Introduction: Fractional laser ablation increases delivery of small molecules, proteins and antibodies into the skin for either local or systemic action but there are no reports of its use to deliver drugloaded carrier systems.

Aims: We describe a "proof-of-concept" investigation to (i) visualize skin deposition of microparticles (MP) following fractional laser ablation using the P.L.E.A.S.E.® (Precise Laser Epidermal System) technology, and (ii) determine the skin biodistribution of triamcinolone acetonide (TA) released from deposited TA-loaded MP (TA-MP).

Methods: Porcine ear skin (thickness ~2 mm) was microporated using the P.L.E.A.S.E.® Er:YAG laser. MP were prepared using poly(DL-lactide-co-glycolide) (Resomer® RG 503H) with (i) fluorescein and Nile Red (NR) for confocal laser scanning microscopy (CLSM) visualization, and (ii) TA for the skin biodistribution study. MP suspended in Milli-Q water were applied to the microporated porcine skin mounted on Franz-diffusion cells and release from deposited MP was studied for 30 min or 48 h. TA release from TA-MP was compared with that from a TA suspension (equivalent amounts of TA were applied). Skin distribution of TA was investigated as a function of depth by extracting TA from five 100-µm slices going from the skin surface to a nominal depth of 500 µm. TA was also extracted from the remaining dermis.

Results: MP mean diameter was 86 ± 3 µm. Microscopic observation of skin samples clearly revealed the formation of a micropore array following P.L.E.A.S.E.® treatment. CLSM images (Fig. 1) showed that the MP were indeed deposited in the micropores (visualized by NR fluorescence) and gradually released fluorescein into the surrounding tissue – present throughout the epidermis and upper dermis after 48 h. The encapsulation efficiency and drug loading of TA were 99.9 ± 1.7 % and 9.8 ± 0.2 %, respectively. The biodistribution study indicated that the TA-MP decreased permeation of TA as compared to the TA suspension and provided more uniform TA levels in the different skin layers.

Conclusion: P.L.E.A.S.E.® poration enabled deposition of MP in micropores and controlled release of the drug load into skin demonstrated the potential of this technique for targeted local therapy.

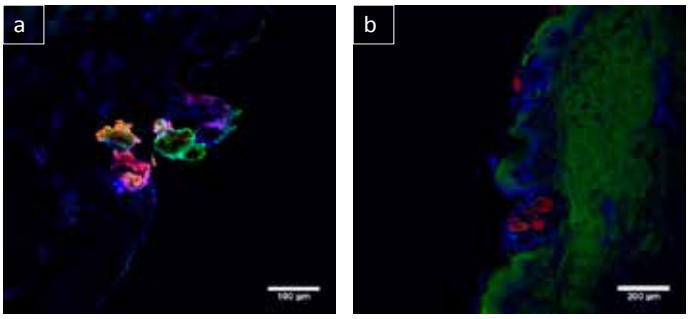

Figure 1 CLSM images (in the XZ-plane) showing fluorescein (green) release as a function of time following (a) 30-min and (b) 48-h application of MP (visualized by the presence of Nile Red fluorescence) in P.L.E.A.S.E.® porated porcine skin (300 pores/cm2, total fluence, 90.6 J/cm2; ~140 µm depth and 150-200 µm diameter). Hoechst blue stain was used to visualize cell nuclei

Keywords: P.L.E.A.S.E.® technology, triamcinolone acetonide, microparticles, skin biodistribution.

P-52

A Polyphenol Enriched Fraction of *Rose Oil Distillation Waste Water* Inhibits Proliferation in Immortalized Human Keratinocytes and Promotes Apoptosis

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Introduction: During the water steam distillation process of rose flowers *(Rosa damascena),* the non-volatile phenolic compounds remain in the waste. While the essential oil depicts the desired liquid for the cosmetic and food industry, the polyphenol containing *rose oil distillation water* (RODW) is in the center of our interest. We recently developed a strategy to separate RODW into a polyphenol depleted water fraction and a polyphenol enriched fraction [RF20-(SP-207)]. Polyphenols are known to have a wide spectrum of biochemical and pharmacological effects, such as antioxidant, anticancer, anti-inflammatory and numerous other activities. In particular, rose petals are known to contain compounds with potential antiproliferative activity, such as flavonoids, gallic and protocatechuic acids, and tannins.
Aims: Up-to-date, medicinal options for diseases showing hyperproliferation of cells such as psoriasis and cancer are strongly limited or utterly insufficient. In this regard, we were interested in the interference between RF20-(SP-207) and cell-proliferation-directing pathways, particularly the Akt/mTOR signaling pathway. Additionally, we examined anti-inflammatory and apoptosis-inducing actions of RF20-(SP-207) in immortalized human keratinocytes (HaCaT).
Methods: All experiments were conducted after a 24-h incubation time. Cell viability was assessed by the MTT-assay. Cell proliferation was elucidated by a commercial BrdU-ELISA assay and the vascular endothelial growth factor (VEGF) as a biomarker of anti-inflammatory response was measured in cell supernatants by a commercial ELISA assay. Apoptotic processes were analyzed by fluorescence microscopy after 488 annexin V conjugate staining. In addition, cells were exposed to UVB radiance to induce apoptosis.
Results: The BrdU-ELISA assay revealed a distinct anti-proliferative activity of RF20-(SP-207) in a dose-dependent fashion. At a concentration of 100 µg/mL, BrdU-incorporation was inhibited by 75% ($IC_{50} \approx 10$ µg/mL). This effect is stronger compared to both positive controls LY294002 10 µM (PI3K-inhibitor, 30% inhibition) and NVP-BEZ235 100 nM (dual PI3K/mTOR-inhibitor, 30% inhibition) and clearly exceeds the anti-proliferative action of quercetin 50 µM (20% inhibition). Cell viability of RF20-(SP-207)-treated keratinocytes decreased up to 50% (100 µg/mL, dose-dependency) compared to the control. In addition, the polyphenol enriched fraction undercut the basic VEGF-level of non-stimulated HaCaT cells significantly, which appeared to be dose-independent. Pro-apoptotic effects of RF20-(SP-207) were illustrated by binding of the 488 annexin V conjugate detected by a fluorescence microscope system. Furthermore, the plant derived fraction did not exert repairing processes on UVB-induced DNA damage.
Conclusions: The polyphenol enriched fraction of *rose oil distillation water* [(RF20 -SP-207)] significantly suppressed cell proliferation and promoted apoptosis in immortalized human keratinocytes. However, the precise mode of action as well as the active compound(s) responsible for the observed effects remains unclear up-until-now. Based on the present data, we hypothesize that RF20-(SP-207) is involved in the Akt/mTOR signaling pathway.

Keywords: *Rosa damascena,* cell proliferation, Akt/mTOR signaling pathway, apoptosis, VEGF.

P-53

Effects of 3,4-Methylenedioxymethamphetamine and Methylphenidate on Circulating Steroid Levels in Healthy Humans

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Introduction: 3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") and methylphenidate (MPH) are widely used psychoactive substances. MDMA primarily enhances serotonergic neurotransmission, and methylphenidate increases dopamine but has no serotonergic effects. Both drugs also increase norepinephrine, resulting in sympathomimetic properties.
Aims: This study was performed to determine the effects of a single dose MDMA (125 mg), MPH (60 mg) or a combination of both on circulating steroid levels in 16 healty volunteers (8 males, 8 females).
Methods: The dosing of the volunteers was performed using a double-blind, randomized, four-session crossover study design. Blood samples were taken at different timepoints up to 24 h after the administration of the drugs. Plasma was prepared and the concentrations of the corticosteroids cortisol, cortisone, corticosterone and 11-dehydrocorticosterone, the mineralocorticoids aldosterone and 11-deoxycorticosterone, as well as the androgens testosterone, androstendione, DHEA and DHEAS were determined by LC-MS/MS.
Results: MDMA significantly increased the plasma concentrations of cortisol, corticosterone, 11-dehydrocorticosterone, and 11-deoxycorticosterone and also tended to increase aldosterone levels compared with placebo. MDMA also increased the sum of cortisol + cortisone and the cortisol/cortisone ratio, consistent with an increase in glucocorticoid production. MDMA did not alter the levels of cortisone, DHEA, DHEAS, androstendione, or testosterone. MPH did not affect any of the steroid concentrations, and it did not change the effects of MDMA on circulating steroids.

Conclusions: In summary, the serotonin releaser MDMA has acute effects on circulating steroids. These effects are not observed after stimulation of the dopamine and norepinephrine systems with MPH. The present findings support the view that serotonin rather than dopamine and norepinephrine mediates the acute pharmacologically induced stimulation of the hypothalamic-pituitary-adrenal axis in the absence of other stressors.

Keywords: MDMA, methylphenidate, steroid, cortisol, aldosterone, testosterone.

P-54

Nanoparticle-Based Drug Delivery to the Brain

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Introduction: The blood-brain barrier (BBB) plays an important role in the homeostasis of the central nervous system (CNS) and prevents entry of most xenobiotics and toxins into the brain. This leads to difficulties in the treatment of CNS related diseases [1]. Biodegradable polymeric micelles, so-called nanoparticles (NPs), are extensively studied as drug carriers. Functionalized NPs can be used to specifically target diseased organs and by this increasing the dose at the site of action, minimizing off-target effects and extending the therapeutic window of the drug. Receptor systems capable to undergo endocytosis were exploited to promote the uptake and transport of drug carrier systems like liposomes or NPs, although the mechanisms behind these systems are not completely understood yet. Recent studies highlighted the transferrin- and the insulin receptor as potential targets for enhanced endocytosis into the brain microvasculature [2,3].
Aims: The aim of this work is to develop a NP-based specific drug delivery system to cross the BBB. This will allow targeting CNS-related diseases using drugs that show no inherent BBB permeability and thus no therapeutic effect in the brain tissue.
Methods: NPs were produced by co-solvent method and analysed by dynamic and static light scattering (DLS/SLS), transmission electron microscopy (TEM) and Cryo-TEM. NPs were functionalized with a monoclonal antibody directed against the human insulin receptor (HIR mAb) using a hetero-bifunctional SM(PEG)-linker.The coupling efficiency and the mAb/NP ratio were determined by fluorescence correlation spectroscopy (FCS). NPs were loaded with the hydrophobic dye DiI to study the *in vitro* performance on a cell based human BBB model (hCMEC/D3) analysed by flow cytometry (FACS).
Results: Self-assembled micelles of biodegradable PEO-b-PCL diblock copolymers had a diameter of 80 nm and showed a narrow size distribution (PDI). The NPs could be covalently modified with a mAB. Quantification using FCS showed 10-15 mAbs/NP. The functionalized NPs showed enhanced uptake into the hCMEC/D3 *in vitro* BBB model as compared to their non-modified counterparts. This effect could be counteracted by competitive inhibition with an excess of free mAB and thus seemed to be specific for the targeted receptor system.
Conclusions: The different uptake kinetics of modified NPs will be analysed on a qualitative level using confocal scanning laser microscopy (CLSM) and TEM. Furthermore, drug loading and release properties of the NPs using doxorubicin as a model drug will be studied using high performance liquid chromatography (HPLC) and *in vitro* based toxicology assays. Finally, *in vivo* experiments will be needed to evaluate the pharmacokinetic and pharmacodynamic properties of the drug delivery system.

Keywords: Blood-brain barrier, nanoparticles, human insulin receptor, drug targeting.

References:
[1] Begley DJ. Pharmacol Therap 2004; 104: 29-45.
[2] Pardridge WM. Pharm Res 2007; 24: 1733-1744.
[3] Pardridge WM. J Cerebral Blood Flow Metab 2012; 32: 1959-1972.

P-55

ICP-133 Controls *In Vitro* Cell Proliferation in Multiple Myeloma Cancer Stem Cells

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Introduction: Despite advances in the development of novel therapies, multiple myeloma (MM) remains an incurable malignancy where the majority of patients relapse, develop resistance and eventually die from the disease. This may be attributed to the fact that conventional therapies mainly target the bulk of tumor cells, but not the tumor-initiating cancer stem cells (CSCs). Thus, tumors composed of heterogeneous cell populations, CSCs and bulk cells, may benefit from combination drug regimens that target each population. However, compounds that target MM-CSCs remain largely unknown. The identification of such compounds is urgently needed as it may significantly improve the prognosis of MM patients. Here we report that ICP-133, a novel anticancer compound, shows promising activity against MM-CSCs.
Aims: The overall aim is to identify novel compounds capable of controlling the growth of MM-CSCs with minimal possible effects to normal stem cells.
Methods: Highly tumorigenic MM-CSCs (CD44+, CD133+, SSEA 3/4+ and OCT4+) were isolated from a newly diagnosed patient with MM. The effects of ICP-133 on cell growth, cell proliferation, cell cycle and apoptosis were assessed on these cells by using the MTT assay, flow cytometric enumeration, DNA staining and annexin V/propidium iodide staining, respectively. The levels of stem cell-related genes were measured by qPCR.
Results: ICP-133 potently inhibited cell growth of MM-CSCs at 72 h, inhibited the proliferation, and induced cell cycle arrest and apoptosis at 24 h in a dose-dependent manner. Quantitative real time PCR analysis of gene products implicated in stem cell maintenance and differentiation revealed that ICP-133 caused an alteration in the expression of Notch1, Notch3, PBX1, HES1, CHUK, NFKBIA, JAG1, GLI2, RUNX1 and SOX2.
Conclusions: The ability of ICP-133 to induce apoptosis and differentially regulate genes involved in stem cell maintenance *in vitro* warrants further investigation in relation to stem cells and potentially in *in vivo* models.

Keywords: Multiple myeloma, cancer stem cells.

P-56

Improving Nutritional Risk Management at a Tertiary Swiss Hospital Center of General Internal Medicine: A Pre-Post Intervention Study

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Introduction: Malnutrition often goes undetected and therefore untreated but has an important impact on clinical outcome. A multicenter survey conducted between 2003-2006 included 32,837 newly admitted Internal Medicine patients of several non-university Swiss hospitals indicated that 18.2% of inpatients were either malnourished or at severe nutritional risk. Therefore, managing patients' nutritional risk is important in the hospital setting [1].
Aims: The goal of the study was to improve malnutrition awareness and management at our department by better educating young physicians. The objectives were to assess whether an online *easylearn* educational program leads to better knowledge of basics in nutrition and as a consequence to beha-vioural changes toward better malnutrition management assessed by the frequency of nutritional therapies (measured by an aimed 10% more prescriptions) [2,3].
Methods: Single centre prospective pre-post intervention study. Nutritional screening (NRS 2002) and prescription of nutritional therapies were assessed in both pre- and post-intervention phases. Parallel, all physicians of the department of general internal medicine had to fill in a questionnaire about basic nutritional knowledge before and after the *easylearn* program (intervention).
Results: 342 patients were included in the pre-intervention and 300 in the post-intervention phase. In the pre-intervention phase 54.09% were at nutritional risk (NRS $\geq$ 3) and in the post intervention phase 61.67%. Only 18.7% of the included patients in the pre-intervention phase and 17.0% of the patients in the post-intervention phase had an adequate nutritional therapy. Forty-nine resp. 41 physicians filled in the questionnaire before and after the intervention. The mean of correct answers was 55.6% resp. 59.4%. Fifty physicians did the *easylearn* program. There was no increase in the prescription of nutritional therapies, induced by the *easylearn* intervention.
Conclusions: Training of the young physician in clinical nutrition is insufficient. The online educational intervention showed only a slight improvement in nutritional knowledge, reflected by the questionnaire filled in and the number of prescriptions of nutritional therapies in patients at risk, despite the high rate of malnourished patients. The *easylearn* 20-30 min online program did not allow to achieve behavioral changes in malnutrition awareness and management in physicians. Further research is needed to improve significantly the knowledge in nutritional basics and the malnutrition management skills of young physicians. Additional well-structured educational and validated didactical activities as bedside teaching, life teaching sessions, clinical skills training, problem based learning, etc. have to be applied.

Keywords: Malnutrition, nutritional risk, educational intervention.

References:
[1] Imoberdorf R et al. Clin Nutr 2010; 29: 38-41.
[2] Mowe M et al. Clin Nutr 2008; 27: 196-202.
[3] Rassmussen HH et al. Clin Nutr 2006; 25: 515-523.

P-57

Home Parenteral Nutrition is Beneficial in Systemic Sclerosis Patients with Gastrointestinal Dysfunction at Risk of Malnutrition

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Introduction: Systemic sclerosis (SSc) is a systemic connective tissue disease which often affects the gastrointestinal tract (GIT). Symptoms caused by GIT dysfunction generally become manifest insidiously, as gastro-oesophageal reflux, pseudo-obstruction, and malabsorption, causing weight loss and subsequent malnutrition [1]. The deterioration of the nutritional and functional status has a severe impact on the prognosis of the underlying disease as well as on quality of life (QoL) and requires an adequate nutritional therapy [2].
Aims: The aim of the present pilot study was to show the benefit of home parenteral nutrition (HPN) on clinical outcome in terms of improvement of nutritional state and QoL during 1 year of observation and to motivate physicians to consider this palliative care with patients at later stages of SSc.
Methods: Observational, retrospective, single-centre, pilot study over 12 months. Data were extracted from paper and electronic medical records. Nutritional risk was assessed based on the internationally validated Nutrition Risk Screening System (NRS 2002). QoL scores were assessed by the SF-36 short form questionnaire.
Results: We studied 5 malnourished patients (no drop-outs) with a mean age of 62.2 years needing HPN. During the 12-months observation period anthropometrics, body function and QoL improved (Physical and Mental Component Summary: PCS: 33.99 vs. 56.52, MCS: 49.66 vs. 88.26 in non-HPN vs. HPN patients, respectively). All components of the SF-36 questionnaire – including physical functioning in everyday life, general health, vitality, emotional and social competence as well as mental health – showed a benefit from HPN. In 2 patients a catheter infection occurred and consequently the i.v. catheter had to be changed, requiring a short hospitalization during the pilot study.
Conclusions: The data show that nutritional support by HPN is a feasible and safe method leading to a health benefit in terms of improved anthropometric parameters and QoL in patients severely affected by SSc and late stages of GIT dysfunction. HPN is strongly recommended for malnourished, catabolic patients unable to maintain their nutritional state by other means.

Keywords: Systemic sclerosis, gastrointestinal dysfunction, malnutrition, home parenteral nutrition, quality of life.

References:
[1] Ranque B, Mouthon L. Autoimmun Rev 2010; 9: A311-8.
[2] Harrison E et al. Gastroenterology 2013; 144: S-749.

P-58

The Influence of Promotional Activities of Pharmaceutical Companies on Prescribing Habits of Physicians in Egypt - A Comparison to the Situation in Western Countries

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Introduction: Pharmaceutical promotion activities in low and middle income countries is often not regulated or monitored or regulated. Egypt has a high population which means a lucrative market for pharmaceutical industry. Most of the pharmaceutical purchases are made out of pockets payments even by the poor and the vulnerable.
Aim: To investigate the influence of the marketing promotional activities of pharmaceutical companies on prescribing habits of physicians in Egypt and compare it to the situation in western countries.
Methods: Semi-structured, in-depth interviews were conducted to explore the perspectives of the different stakeholder, including 20 doctors, 5 pharmacists, 13 pharmaceutical sales representatives, 4 pharmaceutical marketing managers, 2 policy makers and 6 patients (a total of 50 participants). They were chosen via purposive sampling, using also snowball technique and they were interviewed over 4 weeks in Cairo, Egypt. Then thematic analysis was done using NVIVO software.
Results: The majority of doctors and pharmacists believed that some relationship with the pharma-ceutical industry was necessary for performing their professional duties and that there were both risks and benefits associated with it. They considered themselves competent in minimizing the risks and maximizing the benefits. The benefits included support for continuing medical education, scientific journal subscriptions and conference participation. Views diverged on the extent and magnitude of the risks and benefits especially in regards to the influence on patients' health. There is considerable variance in the industry's judgments of what constituted appropriate promotion; while promotion that did not have direct scientific content or benefit to the patient was considered as bribery to influence prescribing.
Conclusions: Pharmaceutical promotion is directed at prescribers and dispensers to maximize profit. Doctors, pharmacists and patients are unaware or skeptical to the influence promotion has towards changing prescribing habits. Patient's welfare if often disregarded in the absence of pharmacovigilance. Monitoring and evaluation of pharmaceutical promotional activities in Egypt is imperative to change the current situation.

Keywords: Pharmaceutical promotion, prescribing habits, pharmaceutical industry, developing countries.

P-59

Oral vs. Intramuscular Vitamin B_{12} Supplementation: Biomarker Response and Patients' Preferences – A Study Design

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Introduction: Supplementation of vitamin B_{12} (VB12) occurs either orally or by intramuscular (i.m.) injection. In Switzerland no high-dose oral mono-preparation is available, despite the evidence of its effectiveness. Non-compliance can impair biomarker response. Electronic monitoring of drug intake can serve to identify patients with deviant compliance and thus to distinguish behavioural aspects from reduced treatment effectiveness. We developed a study design to compare the biomarker responses obtained with oral and i.m. VB12 supplementation under electronically monitored compliance in a representative population in Switzerland. Additionally, patients' preferences between oral and i.m. treatment will be explored.
Aims: To develop a study design to investigate early biomarker responses of oral and i.m. treatment of VB12 deficiency and patient's subjective preferences to the way of administration.
Methods: This study was designed in line with a previous study on "drug resistance" that used electronic compliance monitoring of oral solid drug treatment. Sample size calculation was performed to show non-inferiority of oral to i.m. supplementation. Prescribing information of oral and i.m. VB12 drug forms were analysed to establish treatment schedules. Blood sampling schedules were planned considering endphase distribution kinetics of oral and i.m. administration. A literature search in PubMed was conducted to identify articles focusing on patients' preferences to oral and parenteral therapy options. Questions were generated in collaboration with a clinical psychologist to assess patients' preferences and associated factors at baseline and after study participation.
Results: Prospective randomized unblinded parallel group trial including 60 patients. Patients with serum VB12 concentrations < 200 pmol/l in whom supplementation should be initiated according to the physician's decision will be recruited by their general practitioner. Patients will be randomly assigned to conventional i.m. treatment or to oral treatment. The oral treatment group will be handed out 28 tablets of 1000 µg cyanocobalamin to be taken once daily, prepacked in a 28-day punch card equipped with Polymedication Electronic Monitoring System (POEMS) [1]. Patients in the conventional i.m. group will receive weekly injections of 1000 µg hydroxocobalamin. In both groups, biomarker responses will be assessed at day 0 (baseline), 7, 14, and 28. Patients' preferences will be assessed by a questionnaire including items with 10-point Likert scales and multiple answer options. The questionnaire will be filled out before assignment to oral or i.m. treatment and at day 28. A pilot study with 14 patients demon-strated the feasibility and the reliability of the questionnaire (Cronbach's alpha > 0.66).
Conclusions: The use of POEMS allows controlling for non-compliance as a confounder in studies assessing effectiveness of oral treatments. Early biomarker responses after supplementation with daily oral or weekly i.m. injections can be assessed by 3 blood samplings over 28 days. The developed questionnaire is able to measure changes in patients' preferences and associated factors before and after treatment.

Keywords: Vitamin B_{12} deficiency, patients' preferences.

Reference:
[1] Arnet I, Walter PN, Hersberger KE. Front Pharmacol 2013; 4: 26

P-60

Ligand-Based Pharmacophore Modeling and Virtual Screening for the Discovery of Novel 17β-Hydroxysteroid Dehydrogenase 2 Inhibitors

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Introduction: 17β-hydroxysteroid dehydrogenase (17β-HSD2) belongs to the short-chain dehydro-genase/reductase family of enzymes, converting the active estrogen estradiol into the inactive form estrone. This enzyme is expressed only in a few tissues, such as liver, intestine, placenta and colon. Its inhibition is considered as a target to improve estrogen deficiency.
Aims: In this study we investigated new scaffolds of 17β-HSD2 inhibitors on the basis of ligand based pharmacophore models.
Methods: For the ligand-based pharmacophore modeling, 3 different pharmacophore models were each generated based on 2 different, potent and well known 17β-HSD2 inhibitors. From the literature, 15 active and 30 inactive 17β-HSD2 inhibitors were collected as a test set. Conformations were generated with OMEGA-best settings incorporated in LigandScout 3.03b. Hits were found in the commercial database SPECS (www.specs.net). *In vitro* assays were performed in transfected HEK-293 cell lysates or intact cells using radiolabeled substrates, followed by scintillation counting and quantification of remaining substrate concentration and ascertaining enzyme catalytic activity. All selected hit compounds were initially screened at a concentration of 20 µM.
Results: Models were used for virtual screening of the SPECS database and generated 1531 hit compounds. Following the first virtual screening hits, 29 potential drug-like substances were chosen for *in vitro* 17β-HSD2 inhibition tests. Seven compounds showed strong inhibition effects towards 17β-HSD2 with low micromolar IC_{50} values. The most potent compounds had IC_{50} values of 240 nM and 1.5 µM. To investigate structure-activity relationships (SAR), a further 16 substances were tested. One pharmacophore model outperformed the other 2. This model was therefore used for virtual screening of phenylbenzenesulfonate- or sulfoxy scaffold compounds. A further 14 substances were then tested. These investigations led to identification of 5 compounds with low IC_{50} values, and another potential lead structure with an IC_{50} value of 1 µM. The majority of all hit compounds tested contained either a phenylbenzenesulfonate- or sulfoxy scaffold. The majority of these showed selectivity towards 17β-HSD1 and other related HSD enzymes.
Conclusions: In this study we identified novel 17β-HSD2 inhibitors and potential core structures with potential for treatment of estrogen deficiency.

Keywords: 17β-hydroxysteroid dehydrogenase type 2, inhibitor, pharmacophore modeling, estrogen deficiency.

F-61

Multi-Parametric Evaluation of Therapeutic Protein Aggregation

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Introduction: During the last 30 years the biopharmaceutical industry has grown exponentially, now reaching 140 FDA approved recombinant proteins. However, therapeutic proteins may induce anti-drug antibodies after a few weeks of treatment. Stability problems and aggregate formation are thought to play a major role in this unwanted immunogenicity.
Aims: Using interferon-α2b (IFNα2b) as a relevant example, we aimed to study its stability under various conditions and to decipher the role of therapeutic protein aggregates in the induction of immunogenicity.
Methods: Recombinant human IFNα2b gene was expressed in an *E.coli* BL21(DE3)pLysS-pET28b(+) system. Purification of the protein was done by IMAC and a bioactivity assay was performed with A549 cells infected with vesicular stomatitis virus. The protein structure was characterized in its native state as well as after forced aggregation by circular dichroism (CD), fluorescence spectroscopy and dynamic light scattering (DLS). Aggregation was induced by stirring, metal-catalyzed oxidation, pH modification, thermal stress and freeze-thawing cycles [1].
Results: Protein secondary-structure analysis by CD confirmed its high helical content. After forced degradation we observed a variation in the minimum ellipticity at 220 nm, reflecting structural denaturation of the protein. The 2 tryptophan residues in IFNα2b allowed us to monitor its structural modifications according to the stress applied. Maximum fluorescence emission shifted by 10 nm under thermal stress application, suggesting an increase in the environment polarity or a change in the protein folding. Anisotropy reveals the protein trend to form aggregates after denaturation. With DLS, we were able to estimate the size and subpopulations of aggregates generated. With native protein radii at around 5 nm, thermal stress for example led to big aggregates of up to 2 µm.
Conclusions: Stability studies revealed different aggregation patterns as a result of the stress treatment applied. Combined orthogonal methods gave us a more precise knowledge of the nature of the aggregates.

Keywords: Therapeutic protein, aggregation, immunogenicity.

Reference:
[1] Joubert MK et al. J Biol Chem 2011; 286: 25118-33.

P-62

Efficient Chitosan Carboxymethylation for Biomedical Applications

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Introduction: The application of chitosan to drug and gene delivery is limited due to its poor solubility at physiological pH. An elegant way to enhance its characteristics would be the chemical modification of the chitosan backbone [1]. The chitosan derivative

carboxymethyl-trimethylchitosan (CMTMC) is synthesized from trimethylchitosan (TMC), which shows water solubility over a wide pH range due to its positive charges and highlighted strong adjuvant properties as an excipient for vaccine delivery [2]. CMTMC is also water-soluble at pH >6.5, has a low toxicity and may serve as a scaffold for peptide drug grafting [3]. Still, the application of this material is limited by a low carboxylation grafting efficiency.
Aims: The aim of this study was to optimize the CMTMC synthesis protocol resulting in very high carboxymethyl grafting on TMC.
Methods: TMC synthesis was done through the Eschweiler-Clarke reaction, treating chitosan with formaldehyde in the presence of formic acid, leading to dimethylchitosan (DMC) formation. Later, treating DMC with methyl iodide under weak basic conditions produced TMC. TMC was then suspended in N-methyl-pyrrolidone or isopropanol under stirring at 45°C. Subsequently, 50% NaOH and chloro-acetate were added and left to react for 3 h at 60°C under a nitrogen atmosphere to produce CMTMC.
Results: ^{13}C-NMR spectra displayed characteristic peaks of –COOH groups attached at OH-3 and OH-6 at 71.2 and 71.5 ppm, respectively. ^{1}H-MNR at 4.15-4.5 ppm evidenced successful carboxymethylation, reaching 74% yield and a degree of substitution higher than 85% compared to previously reported carboxymethylation of 15% in OH-6 and 12% in the third position [3].
Conclusions: The obtained results showed a successful carboxymethylation with a high degree of substitution. Such carboxymethylated chitosan could further serve as a starting material for numerous applications such as vaccine and protein or drug delivery in order to enhance cell uptake.

Keywords: Chitosan, chitosan derivatives, vaccine, protein and drug delivery.

References:
[1] Sieval AB et al. Carbohyd Polym 1998; 36: 157-65.
[2] Amidi M et al. J Control Release 2006; 111: 107-16.
[3] Hansson A et al. Carbohyd Polym 2012; 90: 1494-1500.

P-63

Local Delivery of Superparamagnetic Nanoparticles for Application of Local Hyperthermia in Cancer

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Introduction: Prostate cancer shows the third highest mortality of cancerous diseases in men in Europe [1]. Facing the significant side effects of radical prostatectomy or the possibility of active surveillance as common strategies for locally confined prostate carcinoma, alternative treatments to fill this gap are required. Taking into account slow growth of the tumor and main incidence in elderly patients, thermotherapy could provide a therapy regimen without major side effects. Superparamagnetic iron oxide nanoparticles (SPIONs) can dissipate heat when submitted to an external alternating magnetic field, serving as a possible source for local hyperthermia.
Aim: Our intention is to develop an in-situ solidifying nanocomposite containing SPIONs to induce magnetically local hyperthermia as a minimally invasive treatment of prostate cancer. Key requirements for the nanocomposite's application are an appropriate syringeability, radiopacity for real-time monitoring of implant delivery, stability and safety, as well as heating efficacy to reach the threshold temperature of 42°C to induce tumor cell apoptosis [2].
Methods: Poly (vinyl alcohol) covalently grafted with iodinated benzoate radiopaque moieties was chosen as water-insoluble polymer. SPIONs embedded in mesoporous silica were suspended in a DMSO solution of the radiopaque polymer. Rheological behavior of the liquid formulation was assessed using the cone-plate configuration. Upon contact with aqueous solution, the polymer precipitates forming a semi-solid implant, entrapping the silica SPION beads. *In vitro* safety assays of the polymer were performed on PC3 prostate cancer cells and fibroblasts using the cell proliferation reagent WST-1. Cell viability was determined in comparison to non-treated cells. Heating capacity was evaluated by measuring the temperature increase of the implant applying alternating magnetic fields and frequencies which are clinically accepted.
Results: Rheological studies revealed Newtonian behavior of the formulation and a viscosity ensuring appropriate syringeability without any evident risk of phase separation during the injection process. No leakage of silica SPION beads out of the nanocomposite was observed during *in vitro* injection. Based on the threshold of 80% cell viability in comparison to non-treated cells, no cytotoxicity of the polymer could be shown. Application of alternating magnetic fields provoked SPION-induced, frequency- and magnetic field-dependent temperature increase of the implant. The threshold temperature of 42°C for cytotoxicity could be exceeded *in vitro*.
Conclusions: The formulation of silica beads embedding SPIONs suspended in a radiopaque polymer solution shows an adequate syringeability and forms a homogeneous, semi-solid implant upon contact with aqueous solution. Showing no toxic effects of the polymer as carrier material, application of the implant is considered as safe, but has to be confirmed *in vivo*. The entrapped SPIONs are keeping their ability to dissipate heat for local tumor thermotherapy.

Keywords: SPION, *in situ* forming implant, hyperthermia, PC3.

References:
[1] Malvezzi M et al. Ann Oncol 2014; 25: 1650-6.
[2] Hildebrandt B et al. Crit Rev Oncol Hematol 2002; 43: 33-56.

P-64

On Prilled Microgels Containing Clay Nanotubes for Oral Delivery of Proteins

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Introduction: While many new therapeutically active macromolecules are being discovered, their potential use is hindered by their poor oral bioavailability. Microencapsulation is a viable method to protect these macromolecules from the GI barriers, thus increasing the bioavailability, and allows their action especially locally [1]. Prilling is a microencapsulation technique that embeds an API in a polymeric microgel. This is formed by dropping a solution of API and polymer in a hardening bath, where polymeric cross-linking occurs [2]. Halloysite (HNT) is a natural inexpensive aluminosilicate clay, which has a hollow nanotubular structure. HNT has shown potential as a drug delivery system by storing APIs in its lumen or by surface modification [3].
Aims: The purpose of this work was to form HNT-loaded microgels by means of prilling for oral administration of proteins. Bovine serum albumin (BSA) was chosen as a model macromolecule to be loaded in the HNTs, and mono-N-carboxymethyl chitosan (MCC) was selected as polymer for the microgel.
Methods: The HNTs were characterised in terms of size, shape, specific surface area, and pore volume. The BSA was loaded onto the

HNTs by mixing both compounds in a buffered solution (PBS pH 6.8). Subsequently, we applied vacuum to remove air from the nanotubes and allow the entrance of BSA in the HNT lumen. The protein content of the loaded HNTs (loading efficiency) was then assessed. Different amounts of loaded HNTs were dispersed in MCC polymeric solutions, which were then prilled in different hardening media containing calcium chloride (water, ethanol, and diethylene glycol monoethyl ether). The microgels were dried in a vacuum oven for 3 h at 40°C and 20 mbar, and then the protein content was analysed (encapsulation efficiency). The microgel morphology was evaluated by laser diffraction and microscope analysis. The BSA was released from HNTs, microgels, and HNT-loaded microgels in test tubes filled with PBS pH 6.8 at 37°C during 3 h. Finally, the protein stability after manufacturing was assessed by spectropolarimetry and fluorescence.

Results: The HNTs showed a hollow nanotubular structure, with internal diameter of 6 to 15 nm, external diameter of ~35 nm, and an average length of 202 nm. The specific surface area and pore volume were 58.5 m^2/g and 29.7 µL/g, respectively. The loading efficiency of the HNTs was shown to increase with the concentration of BSA, up to 25 mg of BSA per gram of HNT. The dried microgels showed different sizes and shapes according to the hardening bath used; ethanol, however, retained sphericity and had the smallest median particle size (272.8 µm). After an initial screening, the optimal formulation was found to be a 5% HNT and 4.5% MCC *(w/v)* solution dropped in ethanol. This allowed an encapsulation efficiency of 57.2%. All samples released >80% of their content within 1 h. However, the release from HNT-microgels was the slowest, owing to the combined effects of MCC and HNTs. The protein was stable throughout the whole manufacturing process, despite presence of potentially denaturing hardening baths and drying of the microgels.

Conclusions: It was possible to prill microgels containing HNTs loaded with a model protein without compromising the macromolecule's structure. Further research is needed to investigate the behaviour of this system in biorelevant media, in order to understand the synergy of the two components.

Keywords: Halloysite, macromolecular drug delivery, oral drug delivery, prilling.

References:

[1] Tran VT, Benoît JP, Venier Julienne MC. Int J Pharm 2011; 407: 1-11.
[2] Whelehan M, Marison IW. J Microencapsulation 2011; 28: 669-88.
[3] Levis SR, Deasy PB. Int J Pharm 2002; 243: 125-34.

P-65

Prilled Microgels in Lipid Dispersions for Oral Delivery of Biologicals

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Introduction: The prilling technique consists of dropping a solution of API and polymer into an aqueous hardening bath. Here the polymeric hardening takes place by ionic crosslinking [1]. This allows the API entrapment inside microgels while applying mild conditions, which is then suitable for microencapsulation of biologicals. To manufacture a final dosage form (soft or hard shell gelatine capsules), the aqueous hardening bath needs removal, which could harm the macromolecule [2].

Aims: The aim of this work is to introduce a prilling method using a lipid-based hardening bath. This non-aqueous system should be suitable for direct filling into capsules, thus forming a final dosage form.

Methods: A solution of bovine serum albumin (BSA) and mono-N-carboxymethylchitosan (MCC) was dropped by means of the vibrating nozzle principle [3]. The droplets were collected in non-aqueous hardening baths containing calcium chloride to allow ionotropic gelling. The hardening baths were formed by a cosolvent, a glyceride, and a complementary excipient. The microgel morphology was evaluated by dynamic image analysis. The BSA encapsulation efficiency (EE) was quantified with Lowry's protein assay. We studied the BSA release using a USP2 apparatus (37°C and 100 rpm) in phosphate buffer pH 6.8. To evaluate their stability, the microgels were kept for 4 weeks in the hardening baths. The microgel leakage over time was measured in terms of EE. The BSA denaturation during the 4-weeks period was analysed by circular dichroism and SDS-PAGE.

Results: The microgels obtained from different hardening baths had average diameters of ~300 µm. Their shape varied from spherical to toroidal, according to the hardening bath composition. All microgels from non-aqueous hardening baths exhibited high encapsulation efficiency (>85%) and showed fast release (>75% within 15 min). The BSA leakage after 4 weeks from the microgels formed in non-aqueous hardening baths was not statistically significant. Neither the circular dichroism profiles nor SDS-PAGE tests showed protein denaturation after one month, except in one system.

Conclusions: We demonstrated that a hydrophilic microgel containing a macromolecule could be formed by prilling in a non-aqueous medium, which would then be suitable for capsule filling. More biopharmaceutical research would be required to further develop this oral delivery approach for macromolecules.

Keywords: Hydrogel, lipid-based dispersions, macromolecular drug delivery, oral drug delivery, prilling.

References:

[1] Whelehan M, Marison IW. J Microencapsulation 2011; 28: 669-88.
[2] Roy I, Gupta MN. Biotechnol Appl Biochem 2004; 39: 165-77.
[3] Zvonar A et al. J Microencapsulation 2009; 26: 748-59.

P-66

Population Pharmacokinetics of the Novel Anthelminthic Tribendimidine in *Opisthorchis Viverrini* Infected Patients in Lao PDR

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Introduction: Opisthorchiasis is caused by the food-borne trematode, *Opisthorchis viverrini,* and affects more than 8 million people in Cambodia, Lao PDR, Thailand and Viet Nam. The disease causes

hepatomegaly, cholangitis and cholangocarcinoma. Praziquantel is the sole drug available to treat the disease. Tribendimidine is a novel anthelminthic, approved for human use by Chinese authorities since 2004. It is rapidly metabolised in the intestine to deacetylated amidantel (dADT) and further to its acetylated metabolite adADT. It displays an efficacy comparable to the standard drug praziquantel. Moreover, it is well tolerated, which makes this drug an interesting alternative to the standard treatment.

Aims: To elucidate the pharmacokinetic parameters of tribendimidine and its metabolites, dADT and adADT, in a large population of *O. viverrini* infected patients.

Methods: Tribendimidine was delivered to 125 patients at a single oral dose of 400 mg. According to a PK optimised sampling scheme, 5 blood drop samples were collected from patient fingertips between 20 min and 30 h after treatment and deposited on filter cards. The dried blood samples were analysed by a validated liquid chromatography coupled to tandem mass spectrometry method. Pharmacokinetic parameters, as mean maximal concentration (C_{max}), time to maximal concentration (T_{max}), half life ($t_{1/2}$) and area under the curve (AUC) of both tribendimidine metabolites were evaluated.

Results: Tribendimidine was quickly metabolised to dADT and adADT and already after 20 min post-treatment, no parent molecule was detectable in dried blood samples. The dADT metabolite reached higher blood concentrations than adADT (C_{max} of 2.7 and 0.8 µg/mL, respectively). The T_{max} values observed were 6.4 h for dADT and 6.3 h for adADT. The elimination half-lives $t_{1/2}$ were 5.8 and 7.6 h for dADT and adADT, respectively. Finally, the AUC of dADT was 28.0 µg/mL*h, while the AUC of adADT was much smaller (7.7 µg/mL*h). Moreover, our data will be further evaluated by PK-PD analysis to identify important covariates and relate PK parameters with treatment efficacy.

Conclusions: To our knowledge, we have for the first time presented the disposition of tribendimidine in a larger population. To date only one pharmacokinetic study of tribendimidine and dADT in 12 healthy volunteers has been performed. Our data will be helpful in the development of tribendimidine as alternative opisthorchicidal drug.

Keywords: Tribendimidine, *Opisthorchis viverrini,* pharmacokinetics, dried blood spots.

P-67

Coupling HPTLC with MALDI-TOF MS for Detection of Flavonoids

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Introduction: Analysis of flavonoids, namely flavonol aglycones and glycosides, is very important for the examination of plant extracts for quality control purposes using HPTLC. Direct coupling of HPTLC and MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) has been so far successfully applied for the analysis of oligosaccharides [1], lipids and their derivatives (among others [2]) as well as tetracycline antibiotics [3]. To our knowledge, analysis of flavonoids from plant extracts with this method has not been reported extensively.

Aims: The aim of this study was to establish an optimal MALDI-TOF MS method including choice of matrix, matrix concentration and deposition of matrix on the chromatographed silica coated aluminium backed TLC plate for analysis of flavonol aglycones and glycosides which are commonly found in plant extracts.

Method: Standardized HPTLC equipment (Camag) used for HPTLC analysis of flavonoids coupled with MALDI-TOF mass detection (Reflex III - Bruker Daltonics) directly from developed HPTLC aluminum backed HPTLC Silica Gel 60 F254 plates 7.5 x 5cm (Merck) using formic acid, water, ethylmethylketone, ethyl acetate (10:10:30:50 *v:v*) sprayed with 2,5-DHB (Sigma Aldrich) 100mg/mL in 70% methanol as matrix using a Bruker Daltonics HPTLC adapter. For parallel visual derivatization detection of flavonoids Neu's reagent combined with PEG has been used.

Results: Results of method development for successful direct HPTLC coupling with MALDI-TOF MS for analysis of rutin, luteolin-7-O-glucoside, apigenin-7-O-glucoside and their aglycones including determination of signal sensitivity are presented. Further, molecular mass scanning of flavonoid bands on the length of an HPTLC separated plant extract of *Soldanella alpina* spiked with rutin, luteolin-7-O-glucoside and quercetin from starting point to development front is demonstrated.

Conclusions: HPTLC separated flavonoid references and flavonoids in the complex mixture of a plant extract could be detected with their correct masses by direct scanning of the thin layer after development from starting point to front with a MALDI-TOF-MS. Known and unknown substance zones were detected with molecular masses of molecules present on the corresponding Rf value. The method adds valuable complementary molecular mass information to HPTLC analysis in a rapid and less solvent consuming manner as opposed to coupling of HPTLC to MS via a liquid extraction interface.

Keywords: MALDI-TOF-MS, HPTLC, flavonoids, medicinal plants quality control.

References:

[1] Dreisewerd K et al. J Am Soc Mass Spectrom 2006; 17: 139-150.
[2] Torretta E et al. Electrophoresis 2014; 35: 1319-28.
[3] Meisen I et al. Anal Bioanal Chem 2010; 398: 2821-2831.

P-68

Preparation and Characterisation of Chito - Oligosaccharides by MALDI-TOF MS and Size Exclusion Chromatography (SEC-MALLS) for Biomedical Applications

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Introduction: Chitosan is a biodegradable natural polysaccharide with extensive applications in biomedical industry (antifungal, drug delivery, gene delivery, surgical adhesives, etc.). Chitosan-derived oligosaccharides (COS) depict even more unprecedented biomedical properties due to their chemical heterogeneity and thus can be regarded as a natural source for biofunctionality. The link between the biomedical properties of chitosan/COS and their chemical heterogeneity in molecular weight (MW), degree of polymerisation (DP) and degree of acetylation (DA) is well postulated, thus making it necessary to define the "ideal" MW, DA or DP contributing to a given biofunctionality.

Methods: Analytical size exclusion chromatography with multi-angle laser light scattering detector (SEC-MALLS) and matrix-assisted

laser desorption/ionization-mass spectrometry (MALDI-TOF MS) were used for the chemical profiling.
Aims: In this study, a mixture of COS with different DP for further screening as cholesterol binding candidates were successfully generated by acidic degradation of chitosan from different geographical regions and comprehensively characterised.
Results: The MW distribution measured by SEC-MALLS indicated decrease in MW from ≈1.2+10^4 to 6.05+10^3 after 7 h acidic degradation depicting depolymerisation of tested original chitosan into respec-tive COS's. The degree of distribution of COS (DP) by MALDI-TOF was between 2 and 32 indicating that chitosan acidic hydrolysates were a mixture of dimers, trimers, tetramers, etc. The reproducibility of MW measurements was shown by comparing SEC and MALDI-TOF MS analyses. Furthermore, ionisation and fragmentation pattern of oligosaccharides as a function of their DA was evaluated for the first time by MALDI-TOF and MS/MS analyses.
Conclusions: Overall, the usefulness of such multi-dimensional approach to chemical characterisation is enormous as each technique provided complementary and accurate information about the chemical heterogeneity of derivatised COS.

Keywords: Chito-oligosaccharides, chemical characterisation, biomaterial.

References:
[1] Bahrke S et al. Biomacromolecules 2002; 3: 696-704.
[2] Nguyen S et al. Carbohydrate Polymers 2009; 75: 528–533.

P-69

In Vitro Phenotyping of Microsomal Cytochromes P450: New Insights with the Cocktail Approach

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Introduction: Cytochromes P450 (CYPs) are the major phase I metabolic enzymes involved in the oxidative biotransformation of xenobiotics. The activity of CYPs has high inter-individual variability due to genetic polymorphisms and/or environmental factors (e.g. diet, drug therapy, toxic agents, etc.) which are part of the individual phenotype. Some of the major isoforms of the CYP superfamily involved in the metabolism of marketed drugs are recognized as highly polymorphic. According to the type of allelic variant affecting these CYPs, genetic polymorphism can significantly alter their metabolic activity, thus modifying the clinical response and/or increasing the risk of drug-drug interactions (DDI). For these reasons, phenotyping approaches are essential to evaluate and/or anticipate the CYPs activities.
Aims: The aim of our work is the development of a reliable *in vitro* cocktail approach to allow a new potential access to personalized medicine investigations and/or to anticipate toxic phenomena.
Methods: A cocktail mixture was elaborated to increase the throughput of *in vitro* phenotyping studies by monitoring several CYPs activities in a single test. The cocktail comprised 8 CYP-specific probe substrates to simultaneously assess the activity of the most important CYPs, namely 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A subfamily. After cocktail incubation in optimized conditions with human liver microsomes (HLM), the substrates and their metabolites were analysed by a generic LC-MS^E method using ultra-high-pressure liquid chromatography coupled with electrospray ionization quadrupole time-of-flight (QTOF) mass spectrometry (MS).
Results: This cocktail approach was applied to generate the CYP phenotypic profile of different allelic variants of HLM and results were in full agreement with CYPs genetic polymorphisms. This approach was successfully used to enhance the background understanding of the behaviour of xenobiotics on CYPs activities. In this context, the developed cocktail approach was applied to assess the impact of several phytochemicals on microsomal CYPs activities.
Conclusions: The presented cocktail approach could be applied to correlate the metabolism rate with genetic polymorphisms of HLMs. In addition, it successfully highlights the impact of several compounds. Therefore, the proposed approach could be used to evaluate any potential DDI and/or toxicological effects.

Keywords: Cocktail approach, microsomes, LC-MS, genetic polymorphism, phytochemicals.

P-70

Taste-Masked Ibuprofen Micropellets Using an Innovative Spouted Bed Continuous Pelletizing Technology

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Introduction: Organoleptic aspects are one of the determining factors influencing the patient compliance in taking oral medicines. Ibuprofen is a strongly bitter tasting drug used for the treatment of fever, pain or rheumatoid arthritis. Its oral administration can compromise the achievement of the intended therapeutic efficacy, especially for pediatric and geriatric patients. To prevent the drug from interacting with the taste buds, taste-masking techniques are often used. Recent trends are getting towards more robust, cost effective techniques, easy to scale-up or working in a continuous manner. The ProCell™ technology (Glatt, Germany) is an innovative fluid bed technology process offering continuous multi-purpose capabilities as granulation, coating, pelletizing and drying.
Aims: In this study, the ability of the Procell™ technology to prepare taste-masked micropellets providing a fast release of ibuprofen was evaluated.
Methods: The unique design of the process air distribution and the processing chamber of the ProCell™ technology results in a highly controlled particle flow pattern. In a so called "spouted bed", particles are fluidized by a flow of upwards streaming process air which enters the processing chamber through slots at the side rather than across a bottom screen. No inert starting beads are required. The final products are removed continuously as a function of particle size, and separated material is re-circulated into the ongoing process. A melt of ibuprofen and carnauba wax was prepared at a 75/25 ratio and sprayed into the processing chamber of the spouted bed unit at 20-25 g/min spray rate. The process air stream was supplied at 25 °C temperature and at 130 m3/h velocity. To obtain suitable product characteristics, micropellets of size <500 µm were selected. The micropellets were analyzed by sieve analysis and dissolution testing (apparatus type II (paddle), 50 rpm; 2 h in 900 mL of buffered phosphate pH 7.2 ± 0.05 at 37 °C ± 0.5 temperature; 200 mg). Taste assessment was performed using the qualified electronic tongue TS-5000Z (Insent Inc., Japan) equipped with seven lipid membrane sensors representing bitterness, sourness, saltiness, umami and astringency with corresponding aftertastes. Bitter sensor responses (mV) of 3 measurements compared

the formulation to pure ibuprofen and a placebo by Principal Component Analysis (PCA).
Results: The ibuprofen taste-masked micropellets are in the 100-400 µm size range with a d50 of about 200 µm during the production period. Mostly <350 µm in size, the micropellets are particularly suitable for oral administration as such small particles are unlikely perceived in the mouth. In a dissolution medium simulating the conditions in the small intestine, the drug release reaches 80 % in the first 30 min and is complete within 2 h meaning that the product provides fast-release characteristics [1]. The PCA map (90 % of the total information by PC-1 and 10 % by PC-2) reveals that the poor tasting ibuprofen and the drug-free placebo can be distinguished by means of PC-1. Moreover, through PC-2, the diversity between the formulation comprising or not the drug can be seen. It becomes obvious that the taste of ibuprofen is masked from the micropelletized formulation as a shift towards the placebo formulation can be seen in the PCA. Following PC-1, the determination of the distances between the taste-masked micropellets, the pure API and the placebos from these results leads to a taste-masking efficiency of about 60 %.
Conlusions: Highly concentrated taste-masked micropellets providing fast release of ibuprofen were successfully developed. The innovative ProCellTM technology succeeded in achieving challenging product qualities, i.e. an efficient taste masking effect without compromising the intended fast drug release, in an easy and continuous manufacturing process [2]. A product with such characteristics may be used for the development of oral dosage forms where palatability is an issue, as e.g. suspension, stick packs and dispersible or orodispersible tablets. The present work may serve for the development of oral medicines requiring specific product features with the possibility to achieve better therapeutic efficacy and economic benefits.

Keywords: Ibuprofen, taste masking, electronic tongue.

References:
[1] Center for Drug Evaluation and Research (CDER). Guidance for Industry: Orally Disintegrating Tablets. US Food and Drug Administration [Online] Dec 2008. Available at: http://www.fda.gov/OHRMS /DOCKETS /98fr/FDA-2007-D-0365-gdl.pdf.
[2] Gerber F, Guhmann M. Taste-masked ibuprofen granules. Patent Application PCT/EP2013/000908, 26.03.2013.

P-71

Development of a FRET-based HTS for the Identification of Lin28/pre-let-7 Interaction Antagonists

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Introduction: The let-7 microRNAs are notable for their role as inhibitors of cell proliferation. In many cancers this function is reverted by oncogenic Lin28, an RNA binding protein which suppresses let-7 biogenesis by binding to a conserved motif (GGAG) present in the terminal loop regions (TLR) of let-7 precursors (pre-let-7) close to the site of cleavage by RNase III Dicer. Thus, Lin28 inhibits let-7 processing and depletes cells of mature let-7.
Aims: Here, we present as part of our ongoing research program addressing RNA drugability the development of a highly sensitive bimolecular FRET (Fluorescence Energy Transfer)-based assay for the identification of Lin28/pre-let-7 interaction antagonists.
Methods: As FRET partners a GFP-labeled Lin28 donor and dye-labeled pre-let-7a-2 acceptors are involved. Assay optimization was addressed by testing various FRET acceptors bearing different dye labelling positions [1].
Results: The prominent FRET (up to 25%) and an excellent Z-factor value measuring statistical effect size allowed assay up-scaling and miniaturization to HTS (High-Throughput-Screening) [2]. The HTS assay proved to be of good sensitivity and was used to screen a library composed of 16000 organic drug-like small molecules with large structure diversity as a source of potential Lin28/pre-let-7 interaction antagonists. Currently, after hit identification and reevaluation cellular testing of a selection of small molecules is ongoing.
Conclusion: Potential antagonists of the Lin28/pre-let-7 system provide a promising approach to control the endogenous let-7 levels in Lin28-dependent cancers and prevent them from loss of mature let-7 microRNA.

Keywords: High-Throughput-Screening, microRNA, FRET, cancer.

References:
[1] Pradere U et al, Angew Chem 2013; 52: 12028-12032.
[2] Roos M, Pradere U, Hall J. Patent No. 1404, filed 2013.

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1-2/14 Die therapeutischen Blutplasmaproteine: Ihre Geschichte, ihre Bedeutung, ihr Patientennutzen und der Beitrag der Schweiz zur Entwicklung dieser wenig bekannten Industrie
Dr. sc. nat. ETH Zürich Ruedi E. Wäger, Vandoeuvres/Genf, Schweiz

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1-2/14 Ein weiteres Buch „Leading Pharmaceutical Operational Excellence – Outstanding Practices and Cases" erschien im Dezember 2013. Braucht es so viele Bücher zum gleichen Thema?
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Teilnehmer an der Round-Table-Diskussion:
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Prabir K. Basu, Ph.D., OPEX and cGMP Consultant, Mount Prospect, Illinois, USA
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1-2/14 Behördliche Anforderungen an die Reinraumtechnik bei der Herstellung steriler Arzneimittel (Teil 1)
Dr. Hans H. Schicht, Zumikon

PHARMACEUTICAL PRODUCTION • BIOTHERAPEUTICS • BLOOD PLASMA PRODUCTS

3/14 The therapeutic blood plasma proteins: History, patient benefits and the role and contributions of Swiss companies to the progress and success of this little known business sector
Ruedi E. Wäger, Ph.D., Vandoeuvres/Geneva

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3/14 AUTOMATICA 2014: Innovationen aus den Bereichen Pharma, Medizin, Laborautomation – Lösungen für Life Science
Rudolf Güdel – ein Schweizer Unternehmer – Mitinitiant der weltgrössten Fachmesse für Automation und Mechatronik
Gespräch mit Rudolf Güdel, Dipl. Ing. ETH, Inhaber und Verwaltungsratspräsident, Güdel Group AG, Langenthal BE

OPERATIONAL EXCELLENCE • BENCHMARK • PROCESS INDUSTRY • FUTURE OF THE PHARMACEUTICAL • INDUSTRY

3/14 A new book titled «Leading Pharmaceutical Operational Excellence – Outstanding Practices and Cases» was published in December 2013.
It is the third of a series dealing with this topic. Do we really need so many books dealing with the same subject?
Round-table discussion between Felix Wüst and the editors of the book Thomas Friedli, Prabir Basu, Daniel Bellm and Jürgen Werani
Prof. Dr. Thomas Friedli, Prabir K. Basu, Ph.D., Daniel Bellm, Dr. Jürgen Werani

HERSTELLUNG • STERILE ARZNEIMITTEL • BEHÖRDLICHE ANFORDERUNGEN • REINRAUMTECHNIK

3/14 Behördliche Anforderungen an die Reinraumtechnik bei der Herstellung steriler Arzneimittel (Teil 2)
Dr. Hans H. Schicht, Zumikon

AUTOMATICA 2014 • PHARMACEUTICALS • MEDICINE • LABORATORY AUTOMATION • ROBOTS

4/14 AUTOMATICA 2014: Innovations in pharmaceuticals, medicine and laboratory automation – solutions for life science
Rudolf Güdel, Swiss entrepreneur, cofounder of the world's largest specialist trade fair for automation and mechatronics
Interview with Rudolf Güdel, Dipl. Ing. ETH, owner and Chairman of Güdel Group AG, Langenthal (canton of Bern)

POLITIK • GESETZGEBUNG • SPITALAPOTHEKEN • HEILMITTEL

4/14 Politische Entscheidungen und ihr Einfluss auf die Spitalapotheken
Dr. pharm. Enea Felice Martinelli, Spitalapotheker, Spitäler Frutigen Meiringen Interlaken AG, Interlaken

SWISS PHARMA
Swiss Journal of the Pharmaceutical Industry
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PHARMAHERSTELLUNG • CONTAMINATION CONTROL

4/14 Contamination Control in der Pharmaherstellung
Fragen und Antworten am PharmaLunch der Schweizerischen Gesellschaft der Pharmazeutischen Wissenschaften (SGPhW) vom 28. März 2014 in Basel
Dr. sc. techn. Hans H. Schicht, Contamination Control Consulting, Zumikon

FLÜSSIG-/FESTTRENNUNG • FILTRATION • PHARMA/MEDIZIN

4/14 Filtration für Pharma/Medizin – Zwei Kernbereiche der Textilverarbeitungsfirma Lanz-Anliker AG in Rohrbach BE
Die Inbetriebnahme eines Reinraums eröffnet neue Möglichkeiten um weiter und vertiefter in den Pharmasektor und den medizinischen Bereich vorzudringen – Neue Dienstleistungen erschliessen neue Märkte
Gespräch mit Peter Hirschi, Inhaber und Geschäftsführer, Lanz-Anliker AG, Rohrbach BE

HERSTELLUNG • NICHTSTERILE ARZNEIMITTEL • BEHÖRDLICHE ANFORDERUNGEN • REINRAUMTECHNIK

4/14 Behördliche Anforderungen an die Produktionsumgebung bei der Herstellung nichtsteriler Darreichungsformen von Arzneimitteln (Teil 1)
Dr. sc. techn. Hans H. Schicht, Contamination Control Consulting, Zumikon

ROCHE • PHARMAVERPACKUNG • LOGISTIK • KÜHLLAGER

5/14 Bei der F. Hoffmann-La Roche Ltd in Kaiseraugst steht eines der fortschrittlichsten Zentren für Sterilproduktion, Pharmaverpackung und Logistik
Mit dem neuen hochmodernen Kühllager wurde die letzte Lücke im bestehenden System aus Produktion, Verpackung, Lagerung, Qualitätskontrolle und Distribution geschlossen
Gespräch mit Jürgen Simons, Head of Warehouse Operations Kaiseraugst, und Hans Reimann, Senior Project Manager, Site OPEX and Project Office, F. Hoffmann-La Roche Ltd, Kaiseraugst

HERSTELLUNG • NICHTSTERILE ARZNEIMITTEL • BEHÖRDLICHE ANFORDERUNGEN • REINRAUMTECHNIK

5/14 Behördliche Anforderungen an die Produktionsumgebung bei der Herstellung nichtsteriler Darreichungsformen von Arzneimitteln (Teil 2)
Dr. Hans H. Schicht, Zumikon (Schweiz)

SOLID/LIQUID SEPARATION • FILTRATION • PHARMACEUTICAL • MEDICAL

5/14 Filtration for the Pharmaceutical/Medical Industry – Two Core Areas of the Lanz-Anliker AG Textile Processing Company in Rohrbach BE
The commissioning of a clean room provides new possibilities for further and deeper penetration into the pharmaceutical and medical sectors – New services develop new markets
Interview with Peter Hirschi, proprietor and managing director of Lanz-Anliker AG, Rohrbach BE

PHARMAZEUTISCHE PRODUKTION • REINIGUNGSANLAGEN

6/14 Reinigungsanlagen für die pharmazeutische Produktion: Strategischer Umzug der Müller AG Cleaning Solutions an den Hauptsitz der Müller-Gruppe in Münchenstein BL
Modernste Fabrikationseinrichtungen und genügend Teststände stehen bereit für die weitere Expansion des Unternehmens und für Grossaufträge
Gespräch mit Peter Müller, Diplomierter Betriebsökonom FH, Delegierter des Verwaltungsrates und CEO der Müller-Gruppe, Münchenstein BL, und Dr.-Ing. Christian Heuer, Managing Director (COO), Müller AG Cleaning Solutions, Münchenstein BL

PHARMAPRODUKTION • BIOTHERAPEUTIKA • PLASMAPROTEINE

6/14 CSL Behring – Weltweit führendes Unternehmen im Bereich Plasmaprotein-Therapeutika: Rettung von Menschenleben und die Verbesserung der Lebensqualität von Menschen mit schweren und seltenen Krankheiten
Der Mutterkonzern CSL Behring investiert mehrere hundert Millionen US-Dollar in eine neue Produktionsstätte für rekombinante Hämophilie-Therapeutika in Lengnau im Kanton Bern
Die offiziellen Medienmitteilungen der CSL Behring AG, Bern, und der Volkswirtschaftsdirektion des Katons Bern vom 22. Mai 2014

ROCHE • PHARMACEUTICAL PACKAGING • LOGISTICS • REFRIGERATED WAREHOUSE

6/14 F. Hoffmann-La Roche Ltd has one of the most advanced sterile production, pharmaceutical packaging and logistics centres at its Kaiseraugst site
The new state-of-the-art refrigerated warehouse fills the last gap in the existing system comprising Production, Packaging, Storage, Quality Control and Distribution
Interview with Jürgen Simons, Head of Warehouse Operations Kaiseraugst, and Hans Reimann, Senior Project Manager, Site OPEX and Project Office, F. Hoffmann-La Roche Ltd, Kaiseraugst

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Pharmazeutische Wissenschaften • Nachwuchsförderung • EPGL • SAPhW

7-8/14 Nachwuchsförderung in den pharmazeutischen Wissenschaften
Ecole de Pharmacie Genève-Lausanne (EPGL) und Schweizerische Akademie der Pharmazeutischen Wissenschaften (SAPhW)
Gespräch mit Prof. Dr. Gerrit Borchard, Genf
Professor, Biopharmaceutical Sciences, Ecole de Pharmacie Genève-Lausanne (EPGL)
Präsident, Schweizerische Akademie der Pharmazeutischen Wissenschaften (SAPhW)
Vice President, European Federation of Pharmaceutical Sciences (EUFEPS)

Pharmazeutische Industrie • Arzneimittelforschung • Chronische Krankheiten

7-8/14 Der demografische Wandel betrifft uns alle
Der demografische Wandel wird die Gesellschaft in den nächsten vier Jahrzehnten weltweit grundlegend verändern. Ob wir damit erfolgreich umgehen können, hängt auch von der Innovationsfähigkeit der Pharmaindustrie ab. Novartis forscht bereits heute verstärkt im Bereich der chronischen Krankheiten, die in Zukunft weiter zunehmen werden.
Dr. Jörg Reinhardt, Verwaltungsratspräsident, Novartis AG, Basel

Medikamente • Impfstoffe • Krankheiten der Armut

7-8/14 Wirksame Substanzen gegen Krankheiten der Armut
Aus dem Forschungsalltag des Schweizerischen Tropen- und Public Health-Instituts (Swiss TPH)
Marcel Tanner (Direktor Swiss TPH)
Lukas Meier (Kommunikation, Swiss TPH)

Arzneimittelforschung • Molekulare Pharmazie • Sichelzellenanämie

9/14 Ein an der Universität Basel zusammen mit der US-Biotechfirma GlycoMimetics, Inc. entdeckter Wirkstoff zur Behandlung von Sichelzellenanämie rückt der Zulassung als Medikament einen weiteren Schritt näher
Klinische Studien der Phase III stehen bevor
Gespräch mit Professor Dr. Beat Ernst, Professor für Molekulare Pharmazie, Universität Basel, Departement für Pharmazeutische Wissenschaften, Pharmazentrum, Basel

Medication • Vaccines • Diseases of Poverty

9/14 Effective substances against diseases of poverty
A look at the day-to-day research conducted at the Swiss Tropical and Public Health Institute
Marcel Tanner (Director, Swiss TPH)
Lukas Meier (Communication, Swiss TPH)

Pharmazeutische Mikrobiologie • CPM-Meeting

9/14 Curriculum für pharmazeutische Mikrobiologie (CPM)
Bericht vom 19. CPM-Meeting in Weimar (D)
Dr. Michael Rieth, Merck Millipore, Darmstadt (D)

Universität Basel • Juristische Fakultät • Life Sciences-Recht

9/14 Zentrum für Life Sciences-Recht der Juristischen Fakultät der Universität Basel – Bestandesaufnahme und Zukunftsperspektiven
Claudia Caderas, BLaw, Hilfsassistierende am Lehrstuhl für Life Sciences-Recht und Immaterialgüterrecht von Herrn Prof. Dr. iur. Dipl.-Biol. Herbert Zech an der Juristischen Fakultät der Universität Basel

Swiss Pharma Science Day 2014

10/14 University of Bern, 20 August 2014
Swiss Academy of Pharmaceutical Sciences (SAPhS)
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SWISS PHARMA: «Mehr als 30 Jahre im Gespräch mit der Pharmazeutischen Industrie der Schweiz» – Live-Interviews der Jahre 1979 bis 2013

Felix Wüst

In unserem Verlag erschien im Gründungsjahr 1979 – neben vier weiteren Titeln – auch die erste Ausgabe der Zeitschrift SWISS PHARMA, Schweizerische Zeitschrift für die pharmazeutische Industrie (ISSN 0251-1673). Der Titel erscheint nunmehr im 36. Jahrgang (2014) und darf trotz Internet weiterhin grossem Interesse begegnen.

Von Anbeginn an haben wir in SWISS PHARMA Live-Interviews mit Spitzenpersönlichkeiten aus der Pharmaindustrie veröffentlicht. Niemand «durfte sich melden». Wir haben ausnahmslos sämtliche Gesprächspartner immer selber ausgewählt. Niemand wurde dafür je honoriert. Alle haben sich ausnahmslos spontan zu den Gesprächen bereit erklärt. Nie hatte es eine Absage gegeben. «Bedingung» für die Interviews war allerdings immer, dass die Gespräche unvorbereitet, eben «full live» stattzufinden hatten. Und so war es, und das war immer ein grossartiges Erlebnis.

Immer wieder erreichten uns Anfragen nach früher erschienenen Interviews, die wir aber leider nicht befriedigend beantworten konnten, war es doch ein Ding der Unmöglichkeit, von allen Heften seit 1979 auch nur 10 oder 20 Exemplare zu lagern. Nun haben wir sämtliche in SWISS PHARMA je erschienenen Interviews mit genauen bibliographischen Angaben aufgelistet (mit Angabe der Seitenzahlen), so dass ein Interessent bei der Zentralbibliothek Zürich bequem und für wenig Geld Fotokopien anfordern kann. Der Verlag stellt ein Verzeichnis aller SWISS PHARMA-Interviews gerne kostenlos in elektronischer Form zur Verfügung. Mit dieser Dokumentation wird auch mitgeteilt, wie man bei der Zentralbibliothek Zürich per E-Mail Fotokopien eines oder mehrerer Interviews anfordern kann. Das ist möglich, weil die Auflistung wie erwähnt jeweils die Seitenzahlen in den betreffenden Heften aufführt, so dass der Interessent exakt jene Druckseiten als Fotokopien anfordern kann, die er benötigt. Die Zentralbibliothek Zürich berechnet sehr vernünftige Preise für diese Fotokopien: Bis zu 20 A4-Seiten pauschal CHF 10.–; jede weitere A4-Seite zu CHF –.50 (50 Rappen). Die Kopien werden per Briefpost und mit Rechnung an den Besteller zugestellt.

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