SWISS PHARMA

SWISS PHARMA SCIENCE DAY 2011
University of Bern
31 August 2011
Swiss Society of Pharmaceutical Sciences (SSPhS)
Swiss Academy of Pharmaceutical Sciences (SAPhS)

Swiss Journal of the Pharmaceutical Industry

Schweizerische Zeitschrift für die pharmazeutische Industrie

Revue suisse pour l'industrie pharmaceutique

Rivista svizzera per l'industria farmaceutica

SSPhS
Swiss Society
of Pharmaceutical
Sciences

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Swiss Academy
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Sciences

10/11

SGPhW
Schweizerische Gesellschaft
der Pharmazeutischen
Wissenschaften

Société Suisse des Sciences pharmaceutiques (SSSPh)
Società Svizzera delle Scienze farmazeutiche (SSSF)
Società Svizra da las Scienzas farmaceuticas (SSSF)
Swiss Society of Pharmaceutical Sciences (SSPhS)

Zweck und Ziele der SGPhW / *Mission of SSPhS*

Die Gesellschaft fördert alle wissenschaftlichen Interessen der schweizerischen Pharmazie.
Zu diesem Zweck übernimmt sie Funktionen einer Akademie und erfüllt ihre Aufgaben in erster Linie durch:

- Zusammenfassung und Unterstützung der Bestrebungen aller nationaler und regionaler Gesellschaften, die sich mit den pharmazeutischen Wissenschaften befassen.
- Pflege nationaler und internationaler wissenschaftlicher Kontakte. Zusammenarbeit mit anderen wissenschaftlichen Gesellschaften.
- Vertretung der pharmazeutischen Wissenschaften in der Öffentlichkeit.
- Kommunikation pharmazierelevanter Erkenntnisse und Informationen aus Wissenschaft, Forschung und Industrie.
- Auszeichnung von Personen, die sich um die pharmazeutischen Wissenschaften verdient gemacht haben.

The society promotes as a principal goal Pharmaceutical Sciences in Switzerland.
For this purpose the society has assumed the function of an academy pursuing the following mission:

- *Unifying and coaching the national and regional societies linked to the discipline of Pharmaceutical Sciences*
- *Promotion of national and international*
- *scientific contacts and of cooperations with other*
- *scientific societies and academies*
- *Public promotion of Pharmaceutical Sciences*
- *Promotion of the communication of eminent pharmaceutical findings and realizations in science, research, development, industry, health care and public society*
- *To award distinguished persons for their merits in Pharmaceutical Science*

Anmeldung für eine Mitgliedschaft / *Registration for an individual membership*

❒ Ja, ich möchte der SGPhW (Schweizerische Gesellschaft der pharmazeutischen Wissenschaften) als Einzelmitglied beitreten. Den Jahresbeitrag von CHF 50.- (Studenten: CHF 25.-) werde ich mit dem zugesandten Einzahlungsschein überweisen. Studenten: Bitte die Kopie der Studienbestätigung (Studentenausweis) beilegen.
Yes, I wish to join the SSPhS (Swiss Society of Pharmaceutical Sciences) as an individual member. I will transfer the annual membership fee of CHF 50.- (students: CHF 25.-) on receipt of the payment slip. We kindly ask students to enclose a copy of the student card.

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farmaceutica

SWISS PHARMA 10/11

SWISS PHARMA 33 (2011) Nr. 10

CONTENTS

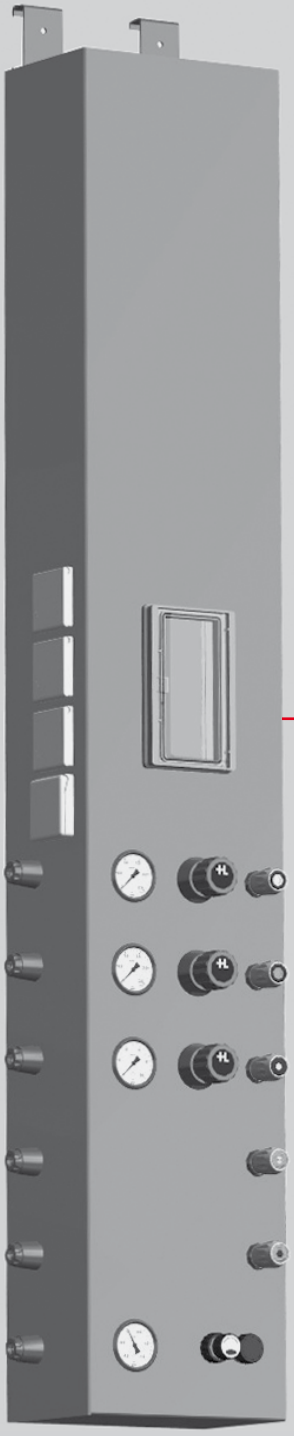
4TH SWISS PHARMA SCIENCE DAY 2011 CONFERENCE REPORT

4TH SWISS PHARMA SCIENCE DAY 2011 POSTER SESSION – PICTURES AND COMMENTS

4TH SWISS PHARMA SCIENCE DAY 2011 KEYNOTE LECTURE

COVER

4TH SWISS PHARMA SCIENCE DAY 2011 POSTER SESSION



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4th SWISS PHARMA SCIENCE DAY 2011

The SWISS PHARMA SCIENCE DAY – now in its 4th year – appears to have acquired the status of an event not to be missed by pharmaceutical scientists all over Switzerland

Prof. Dr. Rudolf Brenneisen, University of Bern, President Swiss Academy of Pharmaceutical Sciences (SAPhS)
Prof. Dr. Gerrit Borchard, University of Geneva, University of Lausanne, School of Pharmaceutical Sciences (EPGL), President Elect Swiss Society of Pharmaceutical Sciences (SSPhS)

The SWISS PHARMA SCIENCE DAY, now in its 4th year, appears to have acquired the status of an event not to be missed by pharmaceutical scientists all over Switzerland.
With an increase of 30% in both participants and poster submissions, the meeting is operating at its capacity limits, as Prof. Rudolf Brenneisen pointed out to a packed lecture hall at the Pathology Building of the University of Bern on August 31, 2011.

Addresses of welcome

Prof. Dr. Rudolf Brenneisen, President SAPhS and organizer of the SPhSD
Prof. Dr. Hans Leuenberger, President SSPhS
Prof. Dr. Peter Eggli, Dean Faculty of Medicine
Prof. Dr. Gerrit Borchard, President elect SSPhS and organizer of SPhSD

Prof. Dr. Rudolf Brenneisen, President SAPhS and organizer of SPhSD.

The day was opened by Prof. Hans Leuenberger, president of the Swiss Society of Pharmaceutical Sciences (SSPhS), and by welcoming the participants and thanking the University of Bern for their hospitality and readiness to host the Swiss Pharma Science Day (SPhSD) again, he pointed out that the structure of this event will be kept. Showcasing research and aspects of all pharmaceutical disciplines, the SPhSD is contributing to keep the pharmaceutical diversity. Same as natural diversity, this can only be a strength of pharmaceutical sciences if it is accompanied by cohesion between the individual disciplines, which the SSPhS and especially the SPhSD is intended to provide.

Prof. Dr. Hans Leuenberger, President SSPhS.

This vision was underlined in the welcome speech by the dean of the Faculty of Medicine at Bern, Prof. Peter Eggli, who welcomed the participants. Prof. Gerrit Borchard, incoming president of SSPhS, announced the Society's new website and logo. The logo, which may be interpreted as four hexagons presenting different pharma-

ceutical disciplines, are converging into a stylized three-dimensional cross representing pharmacy. The website and logo can be viewed at www.sgphw.ch.

Prof. Dr. Peter Eggli, Dean Faculty of Medicine

Prof. Dr. Gerrit Borchard, President elect SSPhS and organizer of SPhSD

The scientific part of the day was opened with a lecture given by Prof. Gerd Folkers of the Collegium Helveticum (ETH Zurich) on the relationship of "Drug design and emotion", or changes in self-perceived role may influence clinical outcome of therapy. A patient may change his self-image by taking a more active role in the therapeutic process, see himself more of an active partner than a "victim" and thereby may influence the final outcome. These changes in self-perceived role may, according to Prof. Folkers, lead to modulation of pain perception, which in itself may be a psychologically constructed effect. The placebo effect of non-steroidal analgesics may find an interesting explanation. The full text of Prof. Folkers' speech is also found in this issue of SWISS PHARMA.

The essential message of Prof. Thomas Kraemer (University of Zurich) was that even though cannabis remains the number 1 drug worldwide, today's drugs such as "spice" are sold as household goods, often from sources in India and China and via internet acquisition and distribution channels. This is why these drugs are often referred to as "Drugs of Abuse 2.0". Spice contains, next to legal exotic herbs, endocannabinoid receptor agonists, which are up to 200 times more potent than tetrahydrocannabinol (THC), and sometimes are not even recognized and listed under the narcotics drug act. This means that they are regarded as legal, and routine drug tests fail to identify these substances. According to Prof. Kraemer, most common drugs in the rave scene are benzyl- and phenylpiperazines, which are taken in combination with anti-emetic drugs and are sold as bath salt and plant food. A repeated drug intake leads to intoxication and death.

The last presentation of the morning was given by Prof. Theo Dingermann of the University of Frankfurt. Citing Hippocrates, he demanded to "treat patients rather than diseases", i.e. to take into account patient factors with regards to their genetic disposition which may be decisive for the success in therapy. In this approach of personalized medicine, diagnostics will be used to reveal the susceptibility of individual patients to selected drugs. These "companion diagnostics" may bear the opportunity to reduce healthcare costs by not wasting budgets on treatment of patients with – for them – ineffective and not tolerated drugs. In other words, a new paradigm of cost effectiveness, based on individual benefit, should be introduced.

In opening the afternoon session, Prof. Giovanni Appendino, of the Faculty of Pharmacy, Università del Piemonte Orientale, proposed that biological agents often show multiple functionalities, a reductionistic view of drug action – effect is therefore not correct. A more sophisticated way in drug discovery, different from a random screening of drug libraries must therefore be applied. In this regard, the systematic examination of natural products for drug candidates might help to "alleviate the shortage" faced by drug discovery and development. Focusing on transient receptor potential (TRP) channels, using a "Flintstone-like" combinatorial chemistry approach ("work like a tinkerer using anything at hand"), he gave the audience a flavor of his field of research.

Following up, Dr. Jacques-Alexis Funel (Actelion Pharmaceuticals, Allschwil) reported on the development of almorexant. The drug belongs to a family of neuropeptide (orexin) receptor antagonists, and was developed for the treatment of insomnia. In particular, Dr. Funel vividly described the implications of the scale-up process from medical chemistry lab to large scale manufacturing, maintaining control of the chiral center contained in the drug's structure. The fact that the drug had to be retracted due to lack of tolerability in patients during a phase III trial added to the real-life experience presented by Dr. Funel.

Dr. Chantal Csajka, researcher in the field of population PK/PD modeling at the School of Pharmaceutical Sciences Geneva-Lausanne (EPGL) and the University Hospital Center of the Canton of Vaud (CHUV), presented the approach of population pharmacokinetic modeling, dependent equally on the pharmacogenetic disposition of the population observed, by discussing the example of methadone. Methadone, a μ-opioid receptor agonist, is used in maintenance-treatment of opioid-dependent patients and for pain treatment. As stereoselective differences for the R- and S-enantiomers are observed, the study focused on identifying genetic and non-genetic causes of both enantiomers' pharmacokinetics, pharmacodynamics and toxicity, and the quantification of the influence of several genes on these properties of methadone.

After a short coffee break it was time for the recognitions and awards. Two outstanding scientists, Prof. Matthias Hamburger and Dr. Felix Wüst were honored with the Fellowship of the Swiss Pharmaceutical Society, and appointed members of the Swiss Academy of Pharmaceutical Sciences.

Then it was time to award the poster prizes. The jury voted unanimously to award the poster contribution of Cristina Fraiz of the School of Pharmaceutical Sciences Geneva-Lausanne the first prize, titled "Injectable organogels to treat tumors through combined hyperthermia and chemotherapy". This was the first time in the history of the SPhSD that an undergraduate student was recognized for work done for a Master's thesis. Evelyne Furger, Paul Scherrer Institute Villigen, was awarded the second prize for her presentation titled "Haptocorrin – The key to vitamin B12 dependent tumour targeting?". The third prize was awarded to Andrea Chicca,

University of Bern, who presented on "Evidence supporting the existence of an endocannabinoid membrane transporter involved in cellular AEA and 2-AG uptake". This year, the jury decided again to award a special poster prize for a fourth outstanding contribution. The poster of Janine Zaugg, University of Basel, titled "Does ethnomedicine-based sample selection increase hit rates in screening of extract libraries? A study on $GABA_A$ receptor modulators" was chosen for this award.The award for the best poster in Pharmaceutical Biology went to Valerie Schuler, University of Zurich, for her contribution "*Bryophyllum pinnatum* press juice – The effect on the contractility of porcine detrusor *in vitro*". Angela Aguilar-de-Leyva, University of Seville, was awarded for the best poster in Pharmaceutical Technology entitled "Study of the properties of a polyurethane polymer (PU(TEG-HMDI)) as an excipient for controlled drug delivery".

Lecture 1: Keynote

Prof. Dr. Gerd Folkers, Collegium Helveticum, ETH Zurich: „Drug design and emotion"

(The Keynote Lecture presented by Prof. Dr. Gerd Folkers is published in full length on page 11 of this issue of SWISS PHARMA.)

Prof. Dr. Gerd Folkers, Collegium Helveticum ETHZ, keynote speaker

Lecture 2: Forensic Pharmacology and Toxicology

Prof. Dr. Thomas Kraemer, Institute of Legal Medicine, University of Zurich: „Drugs of Abuse 2.0: Forget about Cannabis and XTC"

Cannabis is by far the most widely cultivated, trafficked and abused illicit drug. In 2004 around 40% of young people in Europe admitted cannabis use. Four years later a huge drop in lifetime prevalence of cannabis use was reported. However statistics experts calculated that such a decrease in lifetime prevalence is impossible. Different reasons might be responsible for this fact. One reason is quite clear: people do not smoke cannabis any more, they smoke "spice" instead. Spice allegedly contains legal exotic herbs and is sold as an incense. What the ingredients list does not say is that also chemicals are contained. These are endocannabinoid receptor agonists such as JWH-018, -019, -073 or CP47,497 and homologues. Some of them are even more potent (up to 200 times) than tetrahydrocannabinol (THC) from cannabis. Even better for the users: some of them are not yet scheduled drugs under narcotics act, i.e. they are legal. Additional bonus for the "Weedhead 2.0": these substances are not recognized by the police's drug tests. It's only fair that also the "Clubber 2.0" has the choice nowadays. Besides MDMA (ecstasy), there is a wide choice of substitutes such as piperazines or cathinones pharmacologically acting like ecstasy but invisible for today's drug tests. They are such a huge success that ecstasy temporarily had been disappeared from the market. The new drugs are sold as room odorizers, bath salts or plant food – easily available over the internet. The presentation gives an overview over the gray market for spice and new designer drugs. Pharmacological effects, side effects and detection in the modern lab will be discussed.

Prof. Dr. Thomas Kraemer, University of Zurich, in action and receiving a sweet present from Bern

Lecture 3: Translational Medicine

Prof. Dr. Theodor Dingermann, J. W. Goethe University, Frankfurt a.M.: „Diagnostic reaches pharmacy – Treating patients instead of treating diseases" – *"It's far more important to know what person the disease has than what disease the person has"* (Hippocrates)

Currently we are witnessing a paradigm change – some even call it a "tectonic shift" – in medicine: In certain areas, for example in oncology, the "simple" treatment of a disease is slowly but constantly replaced by a treatment of patients with a specific type of disease. This is possible (and necessary), since modern methods of molecular diagnostics allow the collection of data, which provide a precise look into the genetic makeup of a particular patient and his disease. Since the deciphering of the human genome in 2003, the pace of discovery, product development, and clinical adoption of new drugs have been accelerated, which can act only at very specific subtypes of previously as homogeneously recognized diseases. These medicines – if administered to the right patient – will be much more effective and will also be much better tolerated. The consequence is a "personalization of medicine", which may be considered an extension of traditional approaches to understanding and treating a disease, but with much greater precision.

But not only newly developed highly target specific drugs require patient stratification based on molecular diagnostics information. Up to now the individual make up of drug metabolizing capacities of patients was almost completely neglected, with the consequence that many patients did not respond to a guideline conform therapy or they experienced severe adverse drug reaction. Now we can look into the profile of a patient's genetic variation and based on this knowledge drugs or treatment protocols can be selected that minimize harmful side effects and/or ensure a more successful outcome.

We can also identify the patient's susceptibility to certain diseases before they become manifest, allowing the physician and patient to set out plans for monitoring and prevention. If consequently applied and accepted this will more and more shift the emphasis in medicine from reaction to prevention. In addition, these new options will generate also more economic effectiveness and will thus contribute in the efforts to keep high medical standards albeit steadily increasing costs.

Prof Dr. Theodor Dingermann, J.W. Goethe University Frankfurt

Critical questions from the audience

Lecture 4: Pharmaceutical Chemistry

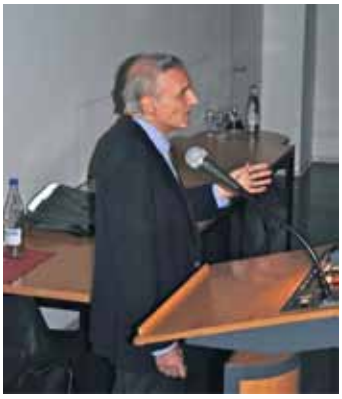
Prof. Dr. Giovanni Appendino, Università del Piemonte Orientale, Novara: „ Adventurous TRPs: Deorphanizing TRPs with small molecule natural products"

The ligand deorphanization of TRP channels has a tremendous potential for biomedical and nutritional research, and its current status exemplifies the role that natural products can play in the identification of ligands for orphan targets and their establishment as viable candidates for drug discovery [1]. Specific small molecules ligands have so far been discovered only for some thermo-TRPs (TRPV1, TRPV3, TRPV4, TRPM8, TRPA1) and for TRPC6, and the lack of selective pharmacology is a major drawback for unraveling the biological role of TRPs. While genetic approaches (transgenic animal models) have partially compensate for the lack of ligands, the success achieved with TRPV1 suggests that a systematic investigation of the natural products pool might alleviate this shortage, fostering adoption within this class of still largely orphan biological targets [2].

References:

[1] J. Vriens, G. Appendino, B. Nilius. Pharmacology of vanilloid transient receptor potential cation channels. Mol Pharmacol 2009; 75: 1262–1279.

[2] C. Avonto, O. Taglialatela-Scafati, F. Pollastro, A. Minassi, V. Di Marzo, L. De Petrocellis, G. Appendino. An NMR spectroscopic method to identify and classify thiol-trapping agents: revival of Michael acceptors for drug discovery? Angew Chem Int Ed Engl 2011; 50: 467–471.

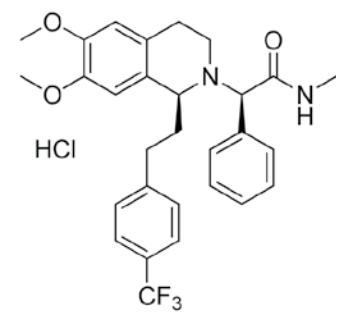


Prof. Dr. Giovanni Appendino, Università del Piemonte Orientale Novara

Lecture 5: Drug Discovery and Development

Dr. Jacques-Alexis Funel, Actelion Pharmaceuticals Ltd, Allschwil: "Almorexant: The first dual orexin receptor antagonist – From discovery to commercial scale manufacturing of drug substance"

Almorexant is a first-in-class orexin receptor antagonist which emerged from Actelion Drug Discovery and was investigated in phase III clinical trial for the treatment of insomnia. The chemistry for its synthesis is presented ranging from early Medicinal Chemistry routes to large scale manufacturing. Emphasis is placed on the development of three alternative, scalable approaches to control the tetrahydroisoquinoline chiral center:



- Racemate resolution using chiral tartranilic acid derivatives.
- Noyori Transfer hydrogenation protocol.
- Use of chiral ferrocenyl ligand Taniaphos.

Details are presented for the route finding and route development activities encompassing ligand screens, cycle time and throughput enhancement.

Dr. Jacques-Alexis Funel, Actelion Pharmaceuticals

Lecture 6: Pharmacology

Dr. Chantal Csajka, University of Geneva and Lausanne, Lausanne: „Population pharmacokinetics, pharmacogenetics and pharmacodynamics of methadone"

Population pharmacokinetic modeling has been recognized over the past two decades as an essential component for accurate description of dose-concentration-effect relationships, determination of optimal dosage regimens, identification of influential covariates and quantification and explanation of variability in drug concentrations and effects among individuals who represent the target population ultimately receiving a drug of interest. It also enables the explanation and quantification of sources of variability in the actual target population ultimately receiving a drug. The recent characterization of genetic polymorphisms implicated in drug kinetics has offered new possibilities for understanding individual patterns of response.

To illustrate this approach, the results of a study characterizing the population pharmacokinetics of methadone are presented. Methadone is a μ-opioid agonist widely used for maintenance treatment in opioid-dependent patients and for pain treatment. It is mainly administered as a racemate mixture of *R*- and *S*-methadone, but stereoselective differences have been reported in their pharmacokinetics, pharmacogenetics and cardiotoxicity. The objectives of the study were to characterize the population pharmacokinetics of *R*- and *S*-methadone in a population of opioid-dependent patients using non-linear mixed effect modeling. It allowed identifying genetic and non-genetic sources of variability on both enantiomers' elimination, while quantifying the respective contribution of several genes involved in the disposition of this drug. A population pharmacokinetic-pharmacodynamic model was built to explore dose-concentration-QT relationships that served to simulate plasma concentrations enabling the prediction of doses associated with an increased probability of QT prolongation.

Dr. Chantal Csajka, University of Geneva and Lausanne

Poster session

During the lunch break with excellent finger food, 69 posters were lifely discussed and carefully examined by the reviewing board members to the tunes of Hello by Martin Solveig & Dragonette and Dear Mr. President by 4 Non Blondies. Like in previous years, awards were given to three outstanding poster presentations, and one poster was given a special award due to its originality. These awards were sponsored by Debiopharm, AKB Foundation and the Pharmaceutical Society of Zurich (first, second and third poster award, respectively), as well as by SSPhS (special award). In addition, the jury was tasked with finding candidates for two more awards, for the best poster in Pharmaceutical Biology (sponsored by Zeller AG) and in Pharmaceutical Technology (sponsored by TTC Glatt AG).

Poster presenters

International attendee

The "Geneva connection" (Proff. Carrupt and Borchard, Dr. Chantal Csajka)

"Has coffee also an addiction potential?"

Prof. Stefan Mühlebach, Vice-President and Fellow SSPhS, and Dr. Isabelle Arnet, University of Basel

Philippe Tschopp and Dr. Christian Lanz, co-organizers SPhSD

Francesca Vollenweider, co-organizer and photographer, and Dr. Felix Wüst, SWISS PHARMA and Fellow SSPhS 2011

Christiane Borchard, photographer

Francesca Vollenweider, co-organizer and photographer

Dr. Maxim Puchov, Univ. of Basel, and Klaus Eichler, TTC Glatt

Recognitions and Awards

Awards waiting for nominees and prize winners

Fellows 2011

Prof. Dr. Matthias Hamburger and Dr. Felix Wüst New Fellows of the Swiss Society of Pharmaceutical Sciences (SSPhS) and new Members of the Swiss Academy of Pharmaceutical Sciences (SAPhS)

Prof. Dr. Matthias Hamburger, pharmacist, was nominated as Fellow by the Swiss Society of Pharmaceutical Sciences (SSPhS) and Member of the Swiss Academy of Pharmaceutical Sciences (SAPhS) for his persistent efforts as head of the Department of Pharmaceutical Sciences, University of Basel, to implement and optimize the pharmacy curriculum according to Bologna and his outstanding research achievements in industry and Swiss as well as foreign universities in the field of Pharmaceutical Biology.

Prof. Dr. Matthias Hamburger, receiving the Fellow Award 2011

Dr. Felix Wüst, publicist, has been elected as Fellow of SSPhS and as Member of the SAPhS for his persistent efforts and dedication to promote the Pharmaceutical Sciences in Switzerland and acting as outstanding banner-bearer for the benefits of the SSPhS and SAPhS. The Swiss Society of Pharmaceutical Sciences, and its Scientific Council, i.e. the Swiss Academy of Pharmaceutical Sciences, are very proud to count Prof. Matthias Hamburger and Dr. Felix Wüst among their ranks.

Dr. Felix Wüst, publisher of SWISS PHARMA, receiving the Fellow Award 2011

Poster award winners

1st Prize, sponsored by Debiopharm:
Cristina Fraiz, School of Pharmaceutical Sciences, University of Geneva-Lausanne
"Injectable organogels to treat tumors through combined hyperthermia and chemotherapy".
Poster P-39

Cristina Fraiz, receiving 1st prize from Dr. Evelyne Vuaridel, Debiopharm

2nd Prize, sponsored by the Foundation of the Association of Bernese Pharmacists (AKB):
Evelyne Furger, Paul Scherrer Institute Villigen
"Haptocorrin – The key to vitamin B12 dependent tumour targeting?"
Poster P-53

Evelyne Furger, receiving 2nd prize from Michele Bordoni, President Association of Bernese Pharmacists (AKB)

3rd Prize, sponsored by the Pharmazeutische Gesellschaft Zurich (PharmGZ):
Andrea Chicca, University of Bern
"Evidence supporting the existence of an endocannabinoid membrane transporter involved in cellular AEA and 2-AG uptake".
Poster P-68

Andrea Chicca, receving 3rd prize from Stephan Dörig, President PharmGZ

Special Prize for originality, sponsored by the Swiss Society of Pharmaceutical Sciences (SSPhS):
Janine Zaugg, University of Basel:
"Does ethnomedicine-based sample selection increase hit rates in screening of extract libraries? A study on $GABA_A$ receptor modulators".
Poster P-9

Janine Zaugg, receiving special prize of SSPhS

Prize for best poster in Pharmaceutical Biology, sponsored by Zeller:
Valerie Schuler, University of Zurich:
"*Bryophyllum pinnatum* press juice – The effect on the contractility of porcine detrusor *in vitro*".
Poster P-59

Coauthor Karin Fürer, receiving Zeller prize

Prize for best poster in Pharmaceutical Technology, sponsored by TTC Glatt Group:
Angela Aguilar-de-Leyva, University of Seville:
"Study of the properties of a polyurethane polymer (PU(TEG-HMDI)) as an excipient for controlled drug delivery".
Poster P-22

Senior author Prof. Isidoro Caraballo, receiving TTC Glatt prize

Thanking … and Invitation to the 5th SWISS PHARMA SCIENCE DAY on Wednesday August 29, 2012

The third Swiss Pharma Science Day ended with drinks and snacks at the beautiful setting of the House of the University. The organizers would like to thank all speakers for their excellent presentations, the poster presenters and coauthors for sharing their scientific data, and the Faculty of Medicine of the University of Bern as the host of this event. The Verlag Dr. Felix Wüst AG Küsnacht, AKB Foundation, Debiopharm, TTC Glatt AG, the Pharmaceutical Society of Zurich (PharmGZ), Vifor Pharma, Mundipharma Medical, Zeller AG, Galexis and pharmaSuisse are recognized for their continued financial support. Last but not least many thanks to all coworkers contributing to the great success of this event.
The organizers are looking forward to welcome young pharmaceutical scientists, academia and pharmacists working in the hospital, industry, public health administration or public pharmacy to the fifth edition of the SPhSD on August 29, 2012, again in Bern.

Members of the organizing committee

Proff. Beat Meier, ZHAW, and Georgios Imanidis, FHNW

Dr. Christian Lanz, co-organizer, and Karin Fürer, poster presenter

Participants enjoying wine and summer evening

4th SWISS PHARMA SCIENCE DAY 2011 (www.sgphw.ch), Bern, 31st August 2011 (Keynote Lecture)

Drug Design and Emotion[1]

Gerd Folkers, Collegium Helveticum, Zurich

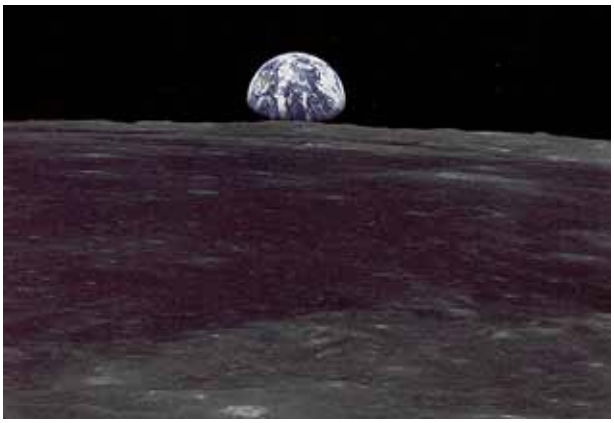
Anyone can become angry – that is easy. But to be angry with the right person, to the right degree, at the right time, for the right purpose, and in the right way – that is not easy.
Aristotle, The Nichomachean Ethics

Emotions and cognition are entangled in a hitherto unknown way. Hence, emotions influence biophysical transcripts and hereby create biochemical reactions on the level of transmitters or hormonal actions, which themselves give rise for physiological response. Neurosciences reveal correlations between synchronized states of neurons and consciousness, cognition, decision making and action. "Emotional tonality" seems to play an important role in these processes and is itself related to language, vision, sense of touch and others. It can be shown that the ambivalence of cognition and emotion is an important factor in individual pain perception and management. Not surprisingly, integrative models of emotion and pain and therapeutic approches are a global cultural heritage. Those facts should be reflected in future drug research.

On Tuesday 25th of July 2006, the Tagesanzeiger in Zurich quoted the Wall Street Journal on a "Glivec Controversy":
"…if further tests should confirm that Glivec may cause heart problems, it might be a drawback for this drug, but as well for all the other 'rational therapeutics', which have been designed to attack cancer cells selectively, and to spare the remaining healthy tissue of the body".
Why is at the same time where drug industry fears yet undetected side effects in the market phase of a new drug as seems to happen in the Glivec case, the most threatening outcome for a new drug in clinical phase II and III is "lack-of-efficacy". Is the increasing frequency of this observation correlated with "lack-of rationality"? Knowing that we will probably never be able to achieve full determinism with respects of the human body and its context interactions, do we approach towards a model though reductionistic, but subtle enough to predict at least cellular behavior at the molecular level? It is in the novel "Simulacron 3", where huge computer technology manages to create virtual worlds of such perfection that simulated mankind in these worlds takes itself for real. This situation sets up the scenario for a fundamental philosophical question that has been addressed since two and half millenniums, still laying unsolved, but being even more virulent today: The problem of subject-object differentiation or the question what does the interior observer share with the exterior one?

The observer's perspective

Let us assume that one would aim for a simulation of living system. The straightforward way would be to construct a Universe based on Hamiltonian reduction.[2] If it has more than two particles, it would encompass an "internal observer" and at the same time being observed from outside. This Hamiltonian Universe would be completely transparent in terms of concepts and notions and would enable a microscopical observation. It seams to make sense, also in terms of physics that the interior observer will perceive his inner world differently from the outer observer.



earthrise. Quelle: http://cygnus.colorado.edu

The creation of the word SUNRISE is a nice example for the situation of an interior and exterior observer. Since it is evident that we see a sunrise every morning, it is also evident that looking at the solar system from outré space, sunrise physically doesn't exist. Or, if you change position, there will be earthrise, instead of sunrise. Inexistent as a physical concept as well. All of that we know for sure since 1543 when Nikolaus Kopernikus published "On the Revolution of Celestial Spheres", dedicated to the Pope Paul III.
The example is of course not fully valid (as every example). It does not consider the important difference of a simulated universe, where there is a creator playing the role of an exterior observer and the (real) evolution based universe, where we create (simulate) an exterior observer: "Weder dem Subjekt noch dem Objekt kommt selbständige Realität zu; jede Existenz beruht auf Wechselwirkung und ist relativ."[3] Nevertheless, the distinction of inner and outer world, to be precise, raises two questions:

1. Do there exist external properties that are not accessible from the inside? (Gödels question or his second incompleteness theorem: *No consistent system can be used to prove its own consistency.*)

1 The paper has been published in part in: AIP Conference Proceedings, November 26, 2007, Vol. 958, pp. 3–8.

2 The simplest interpretation of the Hamilton equations is as follows, applying them to a one-dimensional system consisting of one particle of mass m under time-independent boundary conditions: The Hamiltonian $\mathcal{H}$ represents the energy of the system (provided that there are **NO** external forces, or additional energy added to the system), which is the sum of kinetic and potential energy, traditionally denoted T and V, respectively. Here q is the x coordinate and p is the momentum, mv. Then $\mathcal{H} = T + V, \quad T = \frac{p^2}{2m}, \quad V = V(q) = V(x).$ http://en.wikipedia.org/wiki/Hamiltonian_mechanics

3 Ludvik Fleck (1929:426)

2. Do there exist properties that are valid internally, which are not valid anymore, if the same system is observed from the exterior? (Gedankenexperiment, e.g. Maxwell's Demon, or the EPR)

The reductionistic approach of the Hamiltonian simulation and the up-to-now, completely fictive transformation into a non-classical, life simulating system, has been realized in an ingenious movie "The World on a String" by the late Rainer-Werner Fassbinder in picturizing the novel Simulacron III[4]. But of course the movie tells a story without giving hints for a solution.
However, the movie picks out emotions as a central theme, since two of the simulated subjects (not Simulators) fall in love with each other.
Coming back to the simulation: The concept of quantum mechanics would make the world fundamentally non-deterministic but would not lead necessarily to a "free will", since the great competitor in breaking down the quantum state will be "chance". For an inner observer this might be fate, it occurs, it happens to him.

Edward Burne-Jones Pygmalion. "The soul attains". Quelle: wikipedia public domain

Sharing emotions

Even if it would be possible to make the step towards a non-classical quantum mechanical solution within the simulation which in fact is far from being realized, one would design an interior observer and hence would personalize the emotion in him/her, if at all it would be found there. Like Fassbinder, who elicited in his programmed programmer, being a software construction himself the Pygmalion effect, to fall in love with his artifact, his own construct. Nevertheless Pygmalion experienced shared love at least for the part he constructed and hence shared emotions. If the feeling of shared emotions is true, what we may call empathy, then the same set of emotions belongs to more than one person.[5] Hence it should not be personalized in a constructivistic sense.
This empathy is reflected in the German proverb:
Geteiltes Leid is halbes Leid
Geteilte Freud is doppelte Freud
Obviously we have a cultural tradition in sharing emotion and probably drew evolutionary advantage from it.

This raises the question what sharing of emotions means and what empathy feeling for somebody else, really is. We know from the famous essay *"Wie ist es eine Fledermaus zu sein?"* written by Thomas Nagel that it is impossible to feel like a bat, consciousnessly act like bat and especially share the experience of a bat.[6] If a bat has experience, it will be very probably quite different from ours, it will be alien. Hence we see the situation of an internal observer with his own experiences, the bat, and an external observer, a human researcher, with his measurements and interpretation of a bats experience, which might not match.
Is the world around us and inside of us a mere construction?
If so, should we care about?
We have successfully used the technique of mental construction, to establish all our culture during evolution. Hence it is hard to image, that "those processes of a cognitive rationality, which are so important for our daily life and are an extremely costly part of our life should not have a functional role for the survival of an organism. This would contradict everything that we know about evolutionary processes."[7] Mental construction (inside view?) and empirical position (external observer?) are under this evolutionary point of view necessarily entangled and the mental construction has been an indistinguishable part of the evolution.
As a consequence, Jürgen Habermas claims in his widely recognized response[8], as a consequence, both areas, the empirical one and the mental one, have to observe the world from both perspectives at the same time and in parallel and try to play the role both of the internal and external observer.

Sharing perspectives

We face exactly the same situation as to emotions. I fundamentally criticize, that both fields of expertise, the empirical one (basic sciences) and the mental one (humanities) are on its way to separate emotion from cognition. Of course I may be over exaggerating, but an encyclopedia explains as follows:
"However, Cognitive/behavioral skill building is constructed around mental representations, symbols and the language [words] used to understand and communicate. Emotion, on the other hand is an instinctive response. 'The very root of the word emotion is motere, the Latin verb 'to move', plus the prefix 'e' to connote 'move away…'. 'The emotional/rational dichotomy approximates the folk distinction between 'heart' and 'head' or 'body' and 'mind'; knowing something is right 'in your heart' is a different order of conviction – somehow a deeper kind of certainty – than thinking so with your rational mind."
I hypothesize, and I will give more evidence for that, also in the case of Emotion and Cognition (body and mind,…) the approach of having both perspectives simultaneously is the only valid one and I think the either topic cannot be described without taking into respect the other one. They are complementary to each other and necessarily entangled as well. Let me sum up my view of the whole scenario so far. Obviously there is no clear interface, in the sense of a physical borderline between biochemical processes in the brain and consciousness, but a part, amount unknown, of the biochemical processes in the central nervous system possess the still unexplained peculiarity of being observable from outside, obeys physical laws and in perfect parallelism to manifest itself interiorly as experience, phenomenon, feeling[9]. Hence the truth may both, as so often, particles and waves and has to be considered from both aspects the mental and the empirical one.

4 Daniel F.,Galouye, Simulacron 3, Bantam, New York 1964

5 Tania Singer, Ben Seymour, John O'Doherty, Holger Kaube, Raymond J. Dolan, Chris D. Frith, Empathy for Pain Involves the Affective but not Sensory Components of Pain, Science 303 pp. 1157–1162, 2004

6 Thomas Nagel, What is it like to be a bat?; Philosophical Review 83 (1974): 435–50

7 John Searle, Minds, Brains and Science: The 1984 Reith Lectures (1984)

8 Jürgen Habermas, Um uns als Selbsttäuscher zu entlarven, bedarf es mehr, Frankfurter Allgemeine Zeitung vom 15. Nov. 2004

9 Norbert Bischof, Das Paradox des Jetzt, Psychologische Rundschau, 56, 36–42 (2005)

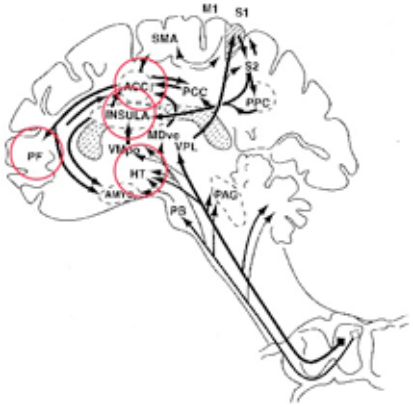
The construtive power of language

Habermas talked about "language games". I'd like to focus now on the example of language and its constructive power with respect to pain, in order to deeper understand the integration of emotion and cognition, both from the interior and exterior point of view.
It is exactly the English language, often scolded for its reductionism and its simplistic attitude. It is however in English that you are able to differentiate between DISEASE and ILLNESS in the description of a suffering person. Looking up the German word LEIDEN in a dictionary you will find several expressions as synonyms like: Sickness, Distress, Disease, Illness, Trouble, Woes. Asking for KRANKHEIT, the French originating MALADY will be added to list. It is evident, and you might feel it that those different expressions have subtle difference in meaning. Why the situation is much more complicated and why it all starts with the choice of a proper wording might be described by a quotation from the Placebo Dialogues, edited by Anne Harrington, Harvard. One of the panel members:
"As a biomedical physician, I like to discriminate between the DISEASE, which is what the physician or the imaging technologies can detect, and the illness, what the patient feels. For me as a gastroenterologist, disease is the stomach ulcer; dyspepsia, its pain, is the ILLNESS. And it is important to realize that there is no tight relationship at all, between the presence of an ulcer crater and the presence of dyspepsia."[10]
This view is strongly supported by the scientific literature.[11]
It is the great English poet, Virginia Woolf who criticizes in deep her own language for its constructivistic inabilities:
"English, which can express the thoughts of Hamlet and the tragedy of Lear, has no words for the shiver and the headache.(…), let a sufferer try to describe a pain in his head to a doctor and language at once runs dry. There is nothing ready made for him. He is forced to coin words himself, and, taking his pain in one hand, and a lump of pure sound in the other (as perhaps the people of Babel did in the beginning), so to crush them together that a brand new word in the end drops out. (…) But it is not only a new language that we need, more primitive, more sensual, more obscene, but a new hierarchy of passions."[12]

Virginia Woolf 1882–1941
Quelle: wikipedia public domain

The perception and expression of pain

This again leads to the interpretation that cognitive processes and emotional processes are tightly entangled. Coining words being a cognitive achievement of molding the "pure sound" of pain with its inherent emotion into a new expression. This makes pain and its expression and consequently its management very much a matter of experience.
I do not talk in this context about the evolutionary necessity of an automatic pain killer, like the endogenous opiod system. It is there, it is necessary for acute pain to keep the body in action, whereas we loose consciousness after a certain threshold. This mechanism is well known since long and has been used in therapy in a manifold of ways, like sexual arousal to ease the pain in mediaeval Japan.
Acute pain may not be the problem in therapy, but pain comes in a plethora of forms, as in rheumatic arthritis, in loosing somebody, in migraine, in fear and anxiety, in cancer diseases. In this contextuality, language, ethnic background, etc. I'd like to come back once more to the placebo issue. It illustrates perfectly the entanglement of emotion and cognition in case of pain management.



Schematic of ascending pathways, subcortical structures, and cerebral cortical structures involved in processing pain.
PAG, periaqueductal gray;
PB, parabrachial nucleus of the dorsolateral pons; VMpo, ventromedial part of the posterior nuclear complex;
MDvc, ventrocaudal part of the medial dorsal nucleus;
VPL, ventroposterior lateral nucleus;
ACC, anterior cingulate cortex;
PCC, posterior cingulate cortex;
HT, hypothalamus;
S-1 and **S-2,** first and second somatosensory cortical areas;
PPC, posterior parietal complex;
SMA, supplementary motor area;
AMYG, amygdala;
PF, prefrontal cortex
Donald D. Price *Science*, **288,** 1769–1772, 2000

"The experience of pain arises from both physiological and psychological factors, including one's beliefs and expectations. Thus, placebo treatments that have no intrinsic pharmacological effects may produce analgesia by altering expectations."[13]
This account acknowledges that pain may be a psychologically constructed experience that includes cognitive evaluation of the potential for harm and affect as well as sensory components. The conclusion would be that PLACEBO effect is an emotionally controlled cognitive process of recognition and intellectual anticipation of potentials.
Let me share with you a last experiment before I summarize:

10 Anne Harrington, The Placebo Effect, An interdisciplinary Exploration. Harvard University Press 2000. P. 211f.

11 Spiegel BM, Vakil NB, Ofman JJ., Dyspepsia management in primary care: a decision analysis of competing strategies. Gastroenterology 122, 1270–85 (2002)

12 Virginia Woolf (1882–1941), On Being Ill, 2002, Paris Press, Ashfield, Mass., 8f.

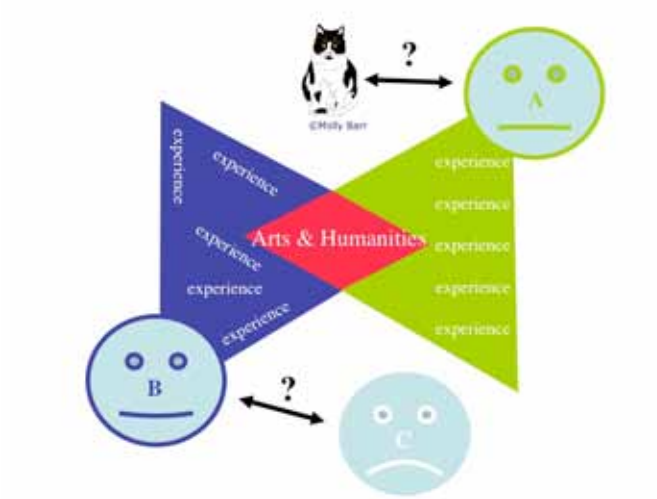
13 Tor D. Wager, James K. Rilling, Edward E. Smith, Alex Sokolik, Kenneth L. Casey, Richard J. Davidson, Stephen M. Kosslyn, Robert M. Rose, Jonathan D. Cohen, Placebo-Induced Changes in fMRI in the Anticipation and Experience of Pain, Science, 303, 1162–1167, 2004

Gaspare Traversi "Die Operation" 1753/54; (left) "Il Ferito" 1752 (right) aus Lit 14.

The scenery in the two paintings of the Napolitano Gaspare Traversi (1732 ca. –1769), are strikingly similar but to the attentive observer, they vividly depict opposing pain sensations in the face of the wounded sufferer: forced and on his own, he grimaces with intense pain, yet facing the proximity of a comforting woman he bravely and calmly endures the awful medical intervention. Apparently, the man perceives himself differently, and this changes his tolerance for the unbearable pain.

Emotional coping with pain

To our knowledge, the effect of self-perceived role identity on pain perception has only been investigated in gender role studies. The effect of different, probably culturally induced, gender-role factors on pain have been shown. For example, coping strategies, pain catastrophizing, situational context, gender role expectations and hyper-vigilance or anxiety have been studied.
Several lines of experimental and daily life evidence indicate that pain sensation is influenced by physiological and psychological factors. Amongst others, gonadal hormones, such as testosterone and oestrogen, athletic status, as well as pain history have been shown to influence pain sensation.
Accordingly, recent studies showed a close connection of brain centers that involve pain and emotions by using functional magnetic resonance imaging (fMRI). Several authors agree that pleasant affective states reduce pain sensation, whereas unpleasant affective states exacerbate it.
We assumed that emotions may be elicited and augmented through the self-perceived role identity and that change of role identity results in a change of emotional state, which in turn changes the intensity of pain perception. More specifically, we assume a) pain can be better tolerated whenever role identity is embedded in an unavoidable, unpleasant context, but which confers pain a meaningful and thus suitable character, and b) that the self-perceived identity changes emotions accordingly, which in turn affects intensity and quality of pain perception. In order to benefit from the strong identification effect during games, and in contrast to other emotions' induction techniques, we used a simplified form of a role-playing game (RPG). By recruiting 21 actors and role players for the study, we aimed at securing the role identification process. The ultimate goal was to establish a learnable self-perceived role identity that can be used not only to ease experimental pain but also in pain therapies.
An increasing body of evidences supports the strong relationship between emotion and pain sensation. Here, we assessed the influence of self-perceived role identity, and its resulting emotional status on both, subjectively and objectively assessed pain sensations. We used role-play strategies to implicitly induce the two antithetic role identities of a *hero/heroine* and a *faint-heart.* We demonstrated that pain tolerance increased when volunteers adopted a *hero/heroine* identity. In contrast, adopting the identity of a *faint-heart* decreased pain tolerance. Furthermore, listening to a scientific text or enduring ten minutes of silence instead of listening to a role-inducing story resulted in decrease of pain tolerance without any changes in sensory or affective scores. Notably, the effect of role identity on pain tolerance was indistinguishable for men and women.[14]
As it has been shown by the example of language and pain, and as it can be shown by imaging/EEG experiments for the different dynamical associations of brain regions in different experiments, emotion and cognition are tightly entangled. This entanglement correlates with the scenario of the external and internal observer, which are entangled as well and where we have to take both positions to learn something about our world.
What remains?



Drug research for integrated therapies

My hypothesis is, that LITERATURE and ARTS, HUMANITIES are the media where the entanglement is reflected. Two persons A and B may be able to share their inner worlds of experience. They create a certain overlap, which may be on the other hand impossible to do with another person C. There may possibly exist other media of exchange with your cat. Artists are able to create physical representations of their inner experience, be it a poem or a painting, the expression of which is both emotionally and cognitively shared by a larger number of people, because it matches parts of their inner experiences.
Therapy should reflect this situation. For instance especially in therapy of chronic pain advantage has been shown for in integrated physical and psychological treatment, but the heavy weight of biomedical research is definitely leaving out inner experience or emotions and their individual uniqueness in the diseased suffering individual.
I refer to the beginning of my talk, where we performed the Gedankenexperiment of a simulation of a Hamiltonian Universe. Research should try to integrate external and internal points of view. Very many of us as we try to make a drug, share the experience that the empiric view alone does not give the expected response. In most cases we blame the bad design of an experiment or the wrong hypothesis, but it may well be that the interior observer does not at all share the same view of his universe, that the empiric exterior observer has developed.

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14 Elvan Kut, Nils Schaffner, Amrei Wittwer, Victor Candia, Meike Brockmann, Claudio Storck und Gerd Folkers, Changes in Self-Perceived Role Identity Modulate Pain Perception, Pain 131 181–190 (2007)

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Poster Session – Abstracts

P-1

Antiplasmodial and Antitrypanosomal Activity of Pyrethrins and Pyrethroids

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Introduction: In a screen of 1800 plant and fungal extracts for antiplasmodial, antitrypanosomal and leishmanicidal activity, the *n*-hexane extract of *Chrysanthemum cinerariifolium* (Trevir.) Vis. flowers showed potent activity against *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense.* At a test concentration of 4.8 µg/mL, the inhibition was 87% and 99%, respectively.
Aims: To identify the compounds responsible for the antiprotozoal activity of a *n*-hexane extract of *C. cinerariifolium,* a series of pyrethrins were isolated and evaluated for their *in vitro* activity and cytotoxicity. Alongside 15 pyrethroids, synthetic analogues of pyrethrins used as insecticidal agrochemicals, were tested for antiparasitic activity.
Methods: Pyrethrin II, jasmolin II, cinerin II, pyrethrin I, and jasmolin I, were isolated from the *n*-hexane extract by medium pressure liquid chromatography and preparative HPLC [1]. The pyrethroids were commercial compounds. The tests against *P. falciparum* (K1 strain), *T. b. rhodesiense* (STIB 900), and for cytotoxicity (rat skeletal myoblast cell line, L-6 cells) were performed by using established protocols [2].
Results: The pyrethrins showed antiplasmodial activity with IC_{50}s between 4 and 12 µM, and antitrypanosomal activity with IC_{50}s from 7 to 31 µM. Pyrethroids exhibited weaker antiplasmodial and antitrypanosomal activity than the pyrethrins. Both pyrethrins and pyrethroids showed moderate cytotoxicity against L6 cells. Pyrethrin II was the most selective antiplasmodial compound, with a selectivity index of 24.
Conclusions: Pyrethrins were the compounds responsible for the antiplasmodial activity of the lipophilic *C. cinerariifolium* extract. These compounds had reasonably selective antiplasmodial properties, and weaker trypanocidal activity. Amongst them, pyrethrin II was the most active and selective compound. In contrast all 15 pyrethroids showed a weaker and less selective inhibition on *P. falciparum* and *T. b. rhodesiense.*

Keywords: *Chrysanthemum cinerariifolium,* pyrethrins, pyrethroids, antiplasmodial, antitrypanosomal.

References:
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[2] M. Adams et al. Nat Prod Comm 2009; 10: 1377-8

P-2

Online Extraction LC-MS^n Method for the Detection of Drugs in Urine, Serum and Heparinized Plasma

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Introduction: In clinical toxicology, specific methods are necessary for the screening of different classes of drugs. For emergency cases, the methods need to be as fast as possible and also as automated as possible to optimize the availability of the method during nights and on weekends.
Aims: An online extraction LC-MS^n method using a MS^2 and MS^3 spectral library for the identification of toxicologically relevant xenobiotic substances has been developed and validated. Since many substances are excreted in urine primarily as metabolites, human liver microsomes (HLM) were used for the generation of metabolites *in vitro,* which were identified using the LC-MS^n method and subsequently added to the library.
Methods: Urine samples were run twice, once native and once after enzymatic hydrolysis. Serum and heparinized plasma samples were run once. Internal standards as well as buffer or acetonitrile were added to urine or serum and heparinized plasma, respectively. Following centrifugation, the supernatant was injected into the system. Extraction was performed by online turbulent flow chromatography. Chromatographic separation was achieved using a phenyl/hexyl 3-µm column. For detection, a linear ion trap, equipped with an APCI interface, was used. The different compounds were identified using a MS^2 and MS^3 spectral library containing more than 450 compounds as well as the retention times.
For the metabolite generation, substances were incubated with the HLM suspension for 3 hours. The reaction was stopped by the addition of acetonitrile. After centrifugation, the supernatant was directly injected into the LC-MS^n system.
Results: The turn-around time to report the results of the screening was less than 1 hour for serum and heparin plasma samples and approximately 2 hours for urine samples including hydrolysis. About 90% of the 450 substances could be identified with a limit of identification below 100 ng/ml in all sample materials. For serum and heparinized plasma, approx. 80% of the substances could be detected already at the lower border of their therapeutic range. A patient sample comparison with existing methods for urine as well as a comparison between screening results in urine and serum or heparinized plasma and urine gave satisfactory results.
Compared with the literature, most metabolites of the tested substances could successfully be identified.
Conclusions: The presented method allows a fast and sensitive analysis of a broad range of compounds in different matrices. Moreover, generation of metabolites and their addition to the spectral library is a very straightforward process.

Keywords: Clinical toxicology, screening, LC-MS, online extraction.

P-3

Genomic Characterization of the Equine Cytochrome P450 3A Gene Family

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Introduction: Cytochrome P450 enzymes (CYPs) represent a superfamily of heme-thiolate proteins with a characteristic reduced carbon monoxide difference spectrum at 450 nm. They play key roles in the metabolism of a variety of substrates including drugs and environmental contaminants. CYPs are most abundant in the liver, a major site of drug metabolism. Interaction of two or more different drugs at the same enzyme can account for unwanted side effects and failure of therapy. Therefore, knowledge about the enzymes responsible for metabolism of a drug is of interest. Human CYP3A4 metabolizes about 30% of all known drugs but little is known about CYPs in horses.
Aim: We report here the genomic organization of the equine *CYP3A* gene cluster as well as a comparative analysis with the human *CYP3A* gene cluster. The equine *CYP* genes of the 3A family are located on ECA13 between 6.99–7.5 Mb in a region syntenic to HSA7 99.2–99.4 Mb. Seven closely linked equine *CYP3A* genes were found, in contrast to four genes in the human genome.
Methods: RNA was isolated from an equine liver sample and the ~1.5 kb coding sequence of six *CYP3A* genes was amplified by RT-PCR.
Results: Sequencing of the RT-PCR products revealed numerous partly hitherto unknown single nucleotide polymorphisms (SNPs) in these six *CYP3A* genes and one 6 bp-deletion compared to the reference sequence on NCBI (EquCab2.0). The presence of the variants was confirmed on the genomic DNA from the same horse and all but one of the variants were found in either heterozygous or homozygous state. A pool of 96 horses was genotyped for two of the polymorphisms. The two tested polymorphisms showed minor allele frequencies of 3,2% and 43%, respectively.
Conclusions: Orthologous genes for the *CYP3A* family exist in horses, but their number differs from the human *CYP3A* gene family. *CYP* genes of the same family show high homology within and between species but can be highly polymorphic. These data provide a basis for functional analyses of the equine *CYP3A* family.

Keywords: *CYP3A* gene family, horse, human, genomic organization, single nucleotide polymorphisms.

P-4

Phenytoin Interacts with 5-Ala Induced PpIX Accumulation in Human Brain Tumor Cells

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Introduction: Tumor-related seizures often appear in patients with glioblastoma multiforme (GBM) which necessitate a treatment with anti-epileptic drugs (AED). For the efficacy of intraoperative fluorescence-guided resection and photodynamic therapy of GBM patients using 5-aminolevulinic acid (5-ALA) to induce accumulation of fluorescent protoporphyrin IX (PpIX), the actual amount of PpIX in the tumor cells is critical.
Aims: The aim of this study was to investigate interactions of two commonly used AEDs, phenytoin and levetiracetam, with 5-ALA induced PpIX synthesis and accumulation in human brain tumor cells.
Methods: Several glioma cell lines and primary GBM cells isolated from tumor biopsies were incubated with AEDs at different concentrations for three days. After treatment with 1mM 5-ALA for 4 hours PpIX accumulation was quantified by fluorescence measurement (photodynamic diagnosis, PDD). For photodynamic therapy (PDT) cells were irradiated with light at 627 nm following 5-ALA treatment and cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Mitochondrial damage was visualized by detecting the mitochondrial membrane potential and confocal laser scanning microscopy.
Results: We observed a decrease in PpIX synthesis of up to 55±12% in primary GBM cells after pre-incubation with high concentrations of phenytoin. This reduction occurred dose- dependently in all tested cell lines and primary GBM cells. Surprisingly, this reduced PpIX accumulation did not affect the efficacy of PDT. Therefore, the integrity of the mitochondrial membrane as location of PpIX synthesis and main target of PDT was investigated. We demonstrated a decrease of the mitochondrial membrane potential after treatment of glioma cells with phenytoin. Levetiracetam affected neither the mitochondrial membrane potential nor PpIX synthesis.
Conclusions: We showed an interaction of phenytoin with 5-ALA induced PpIX synthesis in different glioma cells. We assume that the damage of the mitochondrial membrane by reactive phenytoin metabolites inhibits PpIX synthesis. This premature damage compensates the reduced PpIX accumulation during PDT after phenytoin treatment. In clinical practice 5-ALA induced fluorescence guided surgery could be impaired by long time application of phenytoin.

Keywords: Glioblastoma multiforme, photodynamic diagnosis, photodynamic therapy, 5-amino-levulinic acid, phenytoin.

P-5

Contents of the Ephedrine-Like Alkaloid Synephrine in Traditional Chinese Decoctions

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Introduction: The FDA's ban on *Ephedra* has led to an increase in the use of the ephedrine-like alkaloid synephrine in dietary supplements for the purpose of body loss. Synephrine naturally occurs in bitter-orange (*Citrus aurantium* L.) and other *Citrus* species. Concerns have been raised about the safety of products containing synephrine.
Methods: Tangerine peel (*Citrus reticulata* Blanco; Chenpi) is a herbal drug used in traditional Chinese medicine (TCM) and also contains small amounts of synephrine [1]. Traditional decoctions [2] of this drug are evaluated, i.e. the extraction yields for synephrine in dependence to extraction time. Thereof, an assumed daily intake is calculated for synephrine.
Results: Results showed a content of synephrine of 3.0 mg/g in the herbal drug (batch 1). Traditional decoction resulted in extraction yields of 69% synephrine, referred to dried drug. An extraction profile over time showed similar yields (about 67%) also after 3 h of decoction. Maceration in cold water was about similarly effective, yielding up to 71% of synephrine after 3 h. The analysis of a second herbal drug batch showed a synephrine content of 1.7 mg/g. Traditional decoction resulted in an extraction yield of 75%

synephrine. A longer decoction lasting 2 h led to extraction yields of up to 93%.
Conclusions: Assuming a daily dose of Chenpi of 3–9 g could result in a daily intake of up to 19 mg synephrine. Such doses are below the levels exhibiting pharmacological effects, which are reported to be 100 to 150 mg [3].

Keywords: *Citrus reticulata,* synephrine, decoction, traditional chinese medicine (TCM).

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P-6

Isolation and Characterization of DYRK1A and CLK1 Inhibitors from Plant Extracts

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Introduction: The dual specificity kinase DYRK1A possesses diverse roles in neuronal development and adult brain physiology, and increased activity has been linked to neurodegenerative diseases [1]. Very few inhibitors of this kinase have been reported up to now.
Aims: Discovery of new DYRK1A inhibitors from natural origin.
Methods: A library of plant and fungal extracts was screened against DIRK1A. To identify new inhibitors, the active extracts were submitted to a process integrating physicochemical data with biological information, referred to a HPLC-based activity profiling.
Results: Screening of a library of > 900 plant and fungal extracts afforded 26 extracts with IC_{50}s < 6.2 mg/mL against DYRK1A. Follow-up investigation of three extracts led to the targeted isolation of harmine from *Peganum harmala,* kaempferol and kaempferol-3-O-β-D-galactopyranoside from *Cuscuta chinensis,* and 3,7-dimethyl-herbacetin, 3,8-dimethyl-herbacetin, 3,3',4'-trimethylmyricetin and ombuin from *Larrea tridentata* as the active constituents. Active extracts and compounds were also tested on the closely related kinase CLK1. Harmine and kaempferol show moderate selectivity for DYRK1, with selectivity factors of 4.8 and 3.8, respectively.
Conclusions: The results demonstrate the potential of natural product extracts and HPLC-based activity profiling for the discovery of new DYRK1A inhibitors.

Keywords: DIRK1A kinase inhibitors, HPLC-based activity profiling, flavonoids, harmine.

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P-7

Phytochemical Profiling of Sideroxylon obtusifolium

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Introduction: *Sideroxylon obtusifolium* (Roem. & Schult.) T. D. Penn. (Sapotaceae) is a tree endemic to the Brazilian Sand Coast and Caatinga. Infusions of the bark and the leaves have been used in Brazilian folk medicine as anti-inflammatory agent [1]. Despite its medicinal applications, information on the constituents of *S. obtusifolium* remains scarce, and only common pentacyclic triterpenoids have been previously reported [2].
Aims: Phytochemical profiling of *S. obtusifolium* leaf extracts.
Methods: Leaf extracts of different polarities were analyzed by HPLC-DAD/MS. Targeted isolation of the detected secondary metabolites was subsequently performed by a combination of chromatographic methods including Sephadex LH-20, MPLC and HPLC. The structures of the isolated compounds were elucidated by acid hydrolysis and spectroscopic methods, mainly MS^n, 1D and 2D-NMR experiments.
Results: Saponins and flavonoids were shown to be the main constituents of the leaves. From the butanol-soluble fraction of an ethanolic extract a total of four saponins and ten flavonol glycosides were isolated. They include two new triterpene glycosides 3-*O*-(β-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[D-apiofuranosyl-(1→3)]-α-L-rhamnopyrano-syl-(1→2)-α-L-arabinopyranosyl)-protobassic acid and 3-*O*-(β-D-glucopyranosyl)-28-*O*-(L-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[D-apiofuranosyl-(1→3)]-α-L-rhamno-pyranosyl-(1→2)-α-L-arabinopyranosyl)-protobassic acid, as well as the new flavonol glycosides, quercetin 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside] and kaempferol-3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside]. In addition, catechin and a glycerogalactolipid, gingerglycolipid A, were isolated from the ethyl acetate-soluble fraction.
Conclusions: Oleanane saponins and flavonol glycosides are the main constituents of *S. obtusifolium* leaves. These compounds may be useful as chemical markers for drug quality control.

Keywords: *Sideroxylum obtusifolium,* Sapotaceae, phytochemical profiling, saponins, flavonoids.

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P-8

In Vitro and *in Vivo* Evaluation of a Radioiodinated Folic Acid Conjugate For Folate Receptor Targeted Tumor Imaging

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Introduction: The folate receptor (FR) is overexpressed on a variety of tumor types (e.g. ovarian cancer) but highly restricted in normal tissues with the only exception of the kidneys. FR-targeting with folic acid radioconjugates proved to be a promising concept for nuclear imaging of FR-positive cancer.
Aim: The goal of this study was the development and evaluation of a novel radioiodinated folic acid conjugate useful for nuclear imaging of FR-positive tumors.
Methods: A tyrosine residue was conjugated with folic acid via a "click chemistry" approach resulting in tyrosine-click-folate and the folate conjugate was radiolabeled by direct electrophilic radioiodination (Iodogen®-method) with the ^{125}I-iodine isotope (T1/2=59.4d;

Eg: 35.5 keV, Auger electrons). Stability of the radioiodinated tyrosine-click-folate was investigated in human plasma at 37 °C. FR-binding affinity was determined in a competition assay with FR-positive KB tumor cells using ^{3}H-folic acid and the non-radioactive I-tyrosine-click-folate reference compound. Biodistribution studies were performed in KB-tumor bearing nude mice at 1 h, 4 h and 24 h after injection of the ^{125}I-tyrosine-click-folate. Imaging studies were performed 24 h after injection of the radiofolate using a dedicated small-animal SPECT/CT camera. Additional *in vivo* studies were performed with mice that were preinjected with KI (4 mg; i.p.) and/or with the antifolate pemetrexed (400 µg; i.v.).
Results: ^{125}I-Radioiodination of the tyrosine-click-folate was carried out with a radiochemical yield of > 97%. The ^{125}I-tyrosine-click-folate was stable in human plasma for more than 7 days without detectable deiodination. Binding affinity was high (IC_{50}: 1.5 ± 0.7 nM) and comparable to the value obtained with native folic acid (IC_{50}: 0.9 ± 0.2 nM). The biodistribution data showed FR-specific uptake of ^{125}I-tyrosine-click-folate in tumors (~ 2.3% ID/g, 4 h p.i. and ~ 1.9% ID/g, 24 h p.i). Tumor-to-liver (17.4 ± 9.7, 4 h p.i.) and tumor-to-blood ratios (15.2 ± 4.0, 4 h p.i.) were high already short after injection but undesired accumulation of radioactivity was found in the intestinal tract. A relatively high uptake of radioactivity in the thyroid gland (~ 47.6% ID/g, 24 h p.i.) could be blocked by preinjection of KI. The high uptake of the folate tracer in the kidneys (~ 16.4% ID/g, 4 h p.i. and ~ 7.1% ID/g, 24 h p.i.) as a consequence of FR-specific uptake could be significantly reduced (~ 1.3% ID/g, 4 h and ~ 0.5% ID/g, 24 h p.i.) by administration of pemetrexed 1 h prior to the radiofolate. SPECT/CT imaging allowed excellent visualization of KB tumors in mice 24 h after injection of the ^{125}I-tyrosine-click-folate.
Conclusions: The ^{125}I-radiolabeled tyrosine-click-folate was successfully evaluated as novel imaging agent of FR-positive tumors. Excellent SPECT/CT images were obtained with mice that received KI and pemetrexed. Further investigations will be necessary to evaluate this novel radiofolate for a potential therapeutic application.

Keywords: Folate receptor, tyrosine-folate, iodine, SPECT/CT.

P-9

Does Ethnomedicine-Based Sample Selection Increase Hit Rates in Screening of Extract Libraries? A Study on $GABA_A$ Receptor Modulators

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Introduction: Historically, ethnomedicine-based drug discoveries were based on careful observation of use and effects of specific medicinal plants followed by the isolation of the active principles. Morphine, cardiac glycosides, and ergot alkaloids are only few highlights of this success story. With the paradigm shift in drug discovery to probabilistic high-throughput screening, however, it is unclear whether ethnomedicine-based sample selection can positively contribute to the outcomes of screening campaigns; the available data to this is limited and inconclusive.
Aims: In the context of an extract library screening for new $GABA_A$ receptor modulators, we compared hit rates in different subsets of the library: the entire library (**I**); traditional Chinese medicine (TCM) herbs used in disorders potentially related to $GABA_A$ receptor modulation (e.g. insomnia, panic disorders, epilepsy) (subset **II**); the entirety of TCM herbs in the library (subset **III**).
Methods: Extracts qualified as hit when they showed >100% potentiation of the GABA induced Cl- current at 100 µg/mL in a *Xenopus* oocyte model ($\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptor subtype).
Results: The hit rate of $GABA_A$ receptor modulating extracts in subset **II** was significantly higher than in **I** (0.27 vs. 0.05, p = 0.008). The same was found for subset **III** (0.12 vs. 0.05, p = 0.037). The hit rates in subsets **II** and **III** were not significantly different.
Conclusions: TCM herbs gave a significantly higher hit rate *in vitro* than the entire plant collection. Compounds with scaffolds new for the target were subsequently isolated from **I** and **II,** and pharmacologically characterized *in vitro* and *in vivo*.

Keywords: Ethnomedicine, TCM, $GABA_A$ receptor modulators, *Xenopus* oocytes.

P-10

Rapid Systematic Toxicological Analysis of Micro-Samples of Urine by GC-MS and GC-MS/MS.

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Introduction: In spite of the constant improvement of analytical procedures in term of speed, selectivity and sensitivity, clinical and forensic toxicology is still in need of fast and reliable untargeted screening techniques to identify drugs, poisons and/or their metabolites in biological matrices. Those techniques, designated as Systematic Toxicological Analysis (STA), give essential information for clinical diagnosis, and the ability of giving fast results can be determinant. Urine, when available, remains the most convenient matrix for drug screening, as it concentrates a wide range of drugs and/or their metabolites, and relatively large amounts can be easily and non-invasively collected. Gas chromatography (GC) coupled to mass spectrometry (MS) in full-scan mode is a technique of choice for STA, as it allows a good separation and a reliable identification of analytes of interest. However, sample workup and data handling are time-consuming (up to several hours) and delay the results delivery. GC-MS in full-scan mode may also not be sensitive enough for analytes quantitation.
Aims: The objective of this study is to develop a fast sample preparation procedure for STA in urine, including hydrolysis of glucuronide conjugates, and a fast analysis method, to be able to promptly return a reliable screening of drugs and/or their metabolites in urine.
Methods: Prior to extraction procedure, 50 to 200 µL of urine are hydrolyzed with glusulase under microwave irradiation, during 5 min. Extraction is performed at pH 9 with 1 mL of n-chlorobutane/isopropanol (4/1, v:v). The organic layer is evaporated and acetylated under microwave irradiation, during 5 min, and finally reconstituted in methanol. A 1-µL aliquot is injected on a GC-MS/MS operated in the EI full-scan mode. The run lasts 10 min. Acquired spectra are analyzed by the deconvolution software AMDIS (NIST, version 2.68). Conventional databases are used for spectra comparison and identification. Sample can be re-injected in the same instrument with the appropriate SRM method for confirmation of low concentration compounds and semi-quantitation.
Results: A 5-min irradiation of real urines in a conventional microwave oven is sufficient for a quantitative hydrolysis of glucuronide conjugates of selected model compounds (buprenorphine, benzodiazepines, opiates, THC-COOH). The reduction of urine volumes allows the reduction of solvents and reagents volumes, and consequently the time needed for evaporation. The automated data handling performed by AMDIS showed comparable or even better results than a so-called macro method with manual checking by an experienced technician. Those results were validated by the extraction of 128 real urines. The use of a triple quadrupole mass spectrometer allowed the development of SRM methods for 60 compounds for confirmation at low concentrations and semi-quantitation.
Conclusions: The use of a conventional microwave oven permitted to reduce the time needed for hydrolysis. The use of a sensitive

instrument with an automation of data handling allows an acceleration of sample preparation time, analysis run time and data handling, without impairing the results quality.

Keywords: Urine screening, GC-MS, microwave, automated data handling.

P-11

Ethnobotanical Survey on Wild Food Plants in the Lower and Central Valais

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Introduction: Alpine regions have a rich ancestral tradition with regard to the use of wild plants as medicines and food. However, as a consequence of the far reaching societal and economic changes over the last decades, this traditional knowledge tends to fall into oblivion. This situation prompted us to undertake an ethnobotanical survey on edible wild plants with records of use by the population of the lower and central Valais (Switzerland).
Aims: The aim of this study was to shortlist plants which could potentially be taken into cultivation and re-established as food plants with pleasant sensory properties and a phytochemical composition which might have beneficial effects on health. In the longer run, the project could lead to a new and diversified use of the plant resources of the Alpine regions.
Methods: Informants coming from different valleys of Valais including Entremont, Anniviers and Hérens were interviewed on traditional alimentary uses of wild plants by means of semi-structured questionnaires and interviews. Simultaneously, a literature search in ancient treaties, encyclopedias, and unpublished academic works enabled to identify further plants which were eaten in ancient times by the population of the lower and central Valais.
Results: A total of 107 species of plants distributed in 90 genera belonging to 43 families were identified as used by the alpine population as food. In the investigated regions, wild plants have been mostly used in the form of jams, teas, syrups, liquors, soups and salads. Interestingly, wild edible plants which are still consumed in Valais are mostly reputed to have beneficial long term effects on health. Each region has emblematic plants to which numerous curative properties are ascribed. While older people took their knowledge from the inherited tradition and from childhood's experience in mountain pastures, the younger generation often supplements it with specific courses and popular literature on medicinal plants.
Conclusions: Our ethnobotanical survey demonstrates that the lower and central Valais region has a rich tradition in the use of wild plants. Several species may have a potential for cultivation and commercialization. It is therefore essential to keep and valorize this unique patrimony.

Keywords: Alpine plants, food plants, ethnobotanical survey, Valais, traditional knowledge.

P-12

Phyteumosides A and B: New Saponins with Unique Triterpenoid Aglycons from *Phyteuma orbiculare* L.

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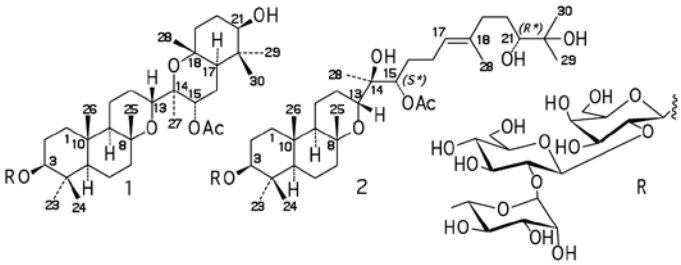
Introduction: The round-headed rampion (*Phyteuma orbiculare* L., Campanulaceae) is a perennial herb which grows in subalpine and alpine regions of Central Europe. The leaves and the flowers were eaten in the past by the population of the Valais region (Switzerland) as a salad. No data have been reported on the secondary metabolites of this species nor the entire genus *Phyteuma*, but plants belonging to the family Campanulaceae are known to contain saponins derived from oleanolic acid.
Aims The aim of this study was to unveil the phytochemical profile of *P. orbiculare.*
Methods: The dried aerial parts were defatted with CH_2Cl_2 and subsequently extracted with MeOH. The MeOH extract was fractionated by a combination of gel filtration on Sephadex LH-20 and flash chromatography on RP-18 (MeOH/H_2O gradient). Structure elucidation of the isolated constituents was performed by spectroscopic and chemical methods including X-ray diffraction analyses.
Results: Two new triterpene glycosides, phyteumosides A (**1**) and B (**2**), were isolated from the aerial parts of *P. orbiculare.* Both compounds possess unique triterpenic aglycons, the structure of which was corroborated by X-ray analyses after enzymatic hydrolysis. The aglycon of **1** can be considered as an incompletely cyclized onoceroid or gammaceroid triterpene with two additional tetrahydropyran rings arising from oxygen bridges. Compound **2** possesses a new 17-polypodene aglycon.



Conclusions: Phyteumosides A (**1**) and B (**2**) possess triterpenoid aglycons with unprecedented cyclisation. Biosynthetically, both aglycons seem to derive from a non-rearranged squalene which underwent incomplete cyclization. Within the Plant Kingdom, gammacerane and onocerane triterpenoids have been mainly found in ferns and club mosses but are rare in seed bearing plants. Compounds **1** and **2** may therefore represent chemotaxonomic markers for the genus *Phyteuma.*

Keywords: Saponins, *Phyteuma orbiculare,* alpine plants.

P-13

Dried Blood Spots Sampling for Clinical and Pharmaceutical Approaches

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Introduction: Over the past decade, dried blood spots (DBS) sampling has emerged as a powerful alternative approach for clinical and pharmaceutical analysis compared to conventional venipuncture procedure. By collecting micro volume of blood after a small finger prick, the DBS process affords numerous advantages that range from better shipment/storage logistics to a less invasive and more ethical sampling procedure [1].
Aims: The recent implementation of DBS sampling process in the University Hospitals of Geneva (HUG) in clinical and toxicological research required an analytical platform to meet these expectations. In this framework, we developed a homemade automate allowing

for the on-line extraction of multiple DBS samples directly into a conventional liquid chromatographic (LC) system with subsequent mass spectrometric (MS) detection. Called "on-line DBS", this procedure allows for the simultaneous analysis of 30 DBS without any sample pretreatment [2].
Methods: Before analysis, DBS samples (i.e. 5 µL) were punched out and set into the homemade prototype to be automatically extracted into the analytical system. The selective desorption of the analytes from the filter paper was carried out by the organic mobile phase toward the reversed phase LC column. After their separation in a gradient elution mode, the analytes were detected using a tandem mass spectrometer (MS/MS) operated in MRM mode with ESI source.
Results: Among the different biomedical approaches tested, the automated on-line DBS concept was first validated and applied to the pharmacokinetic study of flurbiprofen and its metabolite 4-hydroxyflurbiprofen in human volunteers to evaluate the cytochrome P450 2C9 activity. Regarding the good results obtained for the FLB pharmacokinetics, this method has been successfully applied to other clinical and toxicological applications including TDM, target screening, or biomarkers identification.
Conclusions: Associated to selective and sensitive MS/MS detection, the on-line DBS procedure gave good results in the rapid identification and quantification of pharmaceuticals. Combining the advantages of a patient-friendly sampling process with a simple and automated analytical method, this concept is suitable for various biomedical applications.

Keywords: Dried blood spots, direct LC-MS/MS analysis, pharmacokinetics, therapeutic drug monitoring.

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P-14

Impact of the Molecular Lipophilicity Potential (MLP) on Autodock Performance

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Introduction: The molecular lipophilicity potential (MLP) [1] is a molecular interaction field which has found broad application in computational chemistry and ligand-based drug design. It is nowadays routinely used for 3D-QSAR. Further developments in describing the protein cavity lipophilicity showed how MLP can also be applied successfully in target-based methods. For example, the depiction of bindings site hydrophobic areas according to a strict MLP definition of protein cavity hydrophobic areas allowed an overall gain of Genetic Optimisation for Ligand Docking (GOLD) [2] accuracy [unpublished results].
The open source software AutoDock [3] uses pre-calculated 3D grid maps containing energy information about various probe interactions with the binding site. These maps allow rapid evaluations of interaction energies between ligand and protein during docking.
Aims: Embedment of a new MLP term into the map generation process in order to enhance the docking performance of AutoDock. Due to the good overlay of binding site lipophilicity, as defined by MLP and corresponding moieties of the ligand, we are confident about the ability to steer poses towards favorable locations.
Methods: Influencing apolar (carbon) as well as polar (H-bond donor and acceptor) grid maps with respect to the MLP by adding weighted lipophilicity values to the maps.
Conclusions: Docking applying the enhanced grid maps yielded improved binding mode predictions, as observed in individual re-dockings. However, parameterization still requires fine-tuning to maximize the positive influence on docking accuracy. Furthermore these encouraging preliminary results shall be validated on a more extensive ligand-protein structural test set.

Keywords: Lipophilicity, docking, AutoDock, MLP.

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P-15

High-Resolution Ultrasonic Resonator Technology as Novel PAT Tool for Drug Quantification in Self-Emulsifying Drug Delivery Systems

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Introduction: Self-emulsifying drug delivery systems (SEDDS) are complex mixtures of lipidic excipients in which drug quantification is challenging, especially in the context of real-time process analytics.
Aims: To evaluate high-resolution ultrasonic resonator technology as a method for drug quantification in SEDDS.
Methods: The model drugs fenofibrate, indomethacin, and probucol were quantitatively assayed in two different self-emulsifying formulations. We used the ResoScan® System (TF Instruments Inc.) to measure the difference in ultrasound velocity, ΔU, and attenuation, ΔA, between the placebo formulation and the drug-containing formulations (n=33). Linear correlation models were built based on a set of 5–7 calibration samples and the remaining samples were used for model validation. We calculated the limit of quantification (LOQ) and assessed the suitability of the method by means of relative standard error of prediction (RSEP) as well as mean recovery.
Results: Drug quantification using ΔA resulted in relative errors of prediction between 2.3 and 4.4% and LOQs in the range 0.1–0.6% w/w. ΔU provided an interesting analytical response for the quantification of indomethacin. This compound strongly influenced the compressibility of the pure formulation. The LOQ of indomethacin was 0.14 and 0.80% w/w in the two formulations, while the RSEP was below 1.35%. Compared to this result, the quantification of fenofibrate and probucol displayed a slightly lower analytical performance (LOQ 1.2–2.3% w/w; RSEP 3.4–5.4%). These compounds did not strongly affect the density or the compressibility of the pure formulations. Mean recovery values were in the range 98.0–100.6% and 97.0–103.9% for the parameters ΔU and ΔA, respectively.
Conclusions: High-resolution ultrasonic resonator technology proved to be a promising PAT tool in the field of lipid-based systems. Ultrasound attenuation was highly sensitive to drug concentration, independent of the SEDDS components. In contrast, ultrasound velocity was most adequate to quantify drugs that greatly affected the compressibility or the density of the medium.

Keywords: Self-emulsifying drug delivery systems, ultrasonic resonator technology, drug quantification, process analytical technology.

P-16

Development of Orally Available FimH Antagonists for the Treatment of Urinary Tract Infections

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Introduction: In more than 80% of urinary tract infections (UTIs), uropathogenic *Escherichia coli* (UPEC) is the causative pathogen. The initial step in the pathogenesis of the infection is the adherence of UPEC to the human bladder epithelium, enabling the invasion into the host cells. This process is mediated by the lectin FimH located on type I pili enabling UPECs to attach to oligomannosides of the glycoprotein uroplakin Ia presented on uroepithelial cells. FimH antagonists such as α-D-mannopyranosides have been shown to interfere with the attachment of UPEC to their host cells, thus providing a novel therapeutic opportunity for the treatment and prevention of UTIs. However, the pharmacokinetic properties of the tested glycomimetics do not meet the basic requirements of an oral treatment, because they exhibit only insufficient permeability through biological membranes. Since FimH antagonists contain a carboxylic acid moiety, an ester prodrug approach was envisaged to achieve oral availability.
Aims: The aim of this study was the physicochemical and pharmacokinetic characterization of different FimH antagonists and the corresponding methyl ester prodrugs. Further studies focused on the enzymatic hydrolysis of the ester prodrugs, i.e., the release of the active metabolites.
Methods: Lipophilicity was estimated using a miniaturized shake-flask procedure. Thermodynamic solubility measurements were done by an equilibrium shake-flask assay. The parallel artificial membrane permeation assay (PAMPA) was used for an early estimation of the permeability properties. Caco-2 cell based permeability assays were performed to confirm the results from the PAMPA experiments and to study active transport processes. To estimate the metabolic hydrolysis, the ester prodrugs were incubated with rat liver microsomes and the decrease of the prodrug as well as the accumulation of the metabolite were monitored by LC/MS.
Results: The FimH antagonists with a free carboxylic acid were found to be well soluble. However, PAMPA predicted very poor permeability. The corresponding methyl ester prodrugs showed moderate water solubility but the expected high absorption potential by passive permeation. Moreover, permeation studies with Caco-2 cells suggest active efflux by P-glycoprotein. The rate of enzymatic hydrolysis of the methyl esters showed a strong structural dependence. FimH antagonists with a linear shape were more rapidly hydrolyzed than those with a bent structure. Advanced kinetic studies suggest a higher affinity of the linear structures to the carboxylesterase and confirmed the different rates.
Conclusions: The esterification of the free carboxylic acid improved the permeability of FimH antagonists. The rate of the enzymatic release of the active metabolites depends on the molecular structure of the parent compound. Further FimH antagonist prodrugs with modified chemical structures shall be synthesized with the goal to further improve oral availability and to modify the rate of the release of the active metabolites.

Keywords: FimH antagonist, ester prodrug, oral availability, enzymatic hydrolysis.

P-17

Novel High Yield Expression Method for the Production of Recombinant Human Granulocyte Colony Stimulating Factor from *E. coli*

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Introduction: Colony-stimulating factors are haemopoietic proteins that stimulate the proliferation and differentiation of haemopoietic cells from pluripotent stem cells. G-CSF (granulocyte colony-stimulating factor), in particular, stimulates specific bone-marrow precursor cells and their differentiation into granulocytes. Human G-CSF is widely used in hematology and oncology for the amelioration of chemotherapy induced neutropenia, for the restoration of neutrophil production after bone marrow transplantation, for myelodysplastic syndromes, aplastic anemia and severe chronic neutropenia [1]. The recombinant non-glycosylated rhG-CSF produced in *Escherichia coli* is biologically as active as it is when obtained in mammalian cell lines [2]. The E. coli expression system is commonly used for fast production of rhG-CSF at a large scale.
Aims: In this study we have applied a recently developed auto-induction method for the batch expression of rhG-CSF previously cloned into a plasmid under the control of a T7lac promoter [3]. This method, based on a buffered medium that contains a mixture of carbon sources, including lactose, triggers protein expression without the use of costly isopropyl β-D-1-thiogalactopyranoside (IPTG).
Results: We could demonstrate 3-fold higher culture densities and a 3-fold higher protein yield compared to IPTG induction without the need to monitor cell growth in a shortened 24h expression procedure. rhG-CSF expressed in auto-induction media was successfully extracted from E. coli inclusion bodies and refolded by dialysis. After Size Exclusion Chromatography (SEC) purification, rhG-CSF showed conformation, biological activity and aggregation profile similar to the marketed rhG-CSF (Tevagrastim®, TEVA).
Conclusions: Expression by auto-induction is suggested as a cost- and time-effective method for enhanced recombinant human granulocyte-colony stimulating factor production.

Keywords: rhG-CSF, protein expression, neutropenia.

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P-18

Risk Assessment of hERG Channel Inhibition by Natural Products – Activity Directed Analysis of Evodiae Fructus Extract

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Introduction: The most important determinant of acquired long QT syndrome is inhibition of the hERG potassium channel. Drug-induced QT prolongation can cause undesirable cardiac side effects and is thus a liability in drug development. In contrast to synthetic drugs,

herbal medicines are commonly considered to have a better risk-benefit profile. Nevertheless, they may also show adverse effects. Considering the importance of hERG as an antitarget, surprisingly few natural products have been tested for hERG channel blocking properties. Naringenin, the main constituent of grapefruits, is probably the best studied natural hERG blocker, both *in vitro* and *in vivo*.

Aims: To assess the risk potential of natural products on hERG channel inhibition, a wide spectrum of extracts obtained from frequently consumed spices, food, and major medicinal plants were submitted to an *in vitro* screening.

Methods: Currents through hERG channels were studied with transfected *Xenopus laevis* oocytes using the two-microelectrode voltage-clamp technique. For localization and characterization of the bioactivity, we applied a validated HPLC profiling approach that combines HPLC-micro-fractionation with on-line and off-line spectroscopy.

Results: One of the most active samples was a methanolic extract of the TCM herbal drug Evodiae Fructus (*Evodia rutaecarpa* [Juss.] Benth., Rutaceae). When tested at 100 µg/mL, the extract diminished the peak tail hERG current by 60.9 ± 6.9%. HPLC-based activity profiling of the crude extract led to the identification of dehydroevodiamine and hortiamine, two indoloquinazoline alkaloids, as the active principles. Both compounds showed similar hERG channel blocking properties with IC_{50} values in the µM-range (7.4 ± 2.2 µM and 7.9 ± 1.8 µM, respectively). First information on structure-activity relationships revealed that both the methyl group at position 14 and the double bond between C(13b) and N(14) are essential for the inhibitory effect of this compound class. Different commercial batches of Evodiae Fructus and various processed TCM products were analyzed for their dehydroevodiamine content. The daily intake in dehydroevodiamine from crude drugs was significantly higher than from processed herb preparations. To improve the safety profile of herbal extracts from *Evodia,* we designed a procedure for the selective removal of both inhibitory alkaloids. After filtration over a cation exchange resin (Lewatit® MonoPlus SP 112), the resulting extract was devoid of hERG channel blocking activity.

Conclusions: Dehydroevodiamine and hortiamine, two structurally related alkaloids, were identified in the TCM herb *Evodia rutaecarpa* as potent hERG channel blocker. An efficient method for removing both blockers from the extract was developed.

Keywords: hERG channel inhibition, herbal extracts, HPLC-based activity profiling, two-microelectrode voltage-clamp assay on *Xenopus* oocytes, *Evodia rutaecarpa,* indoloquinazoline alkaloids.

P-19

Nanocomplexes of a RGD Peptide-Functionalized Biopolymer for the Promotion of Cell Migration

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Introduction: On wounding, keratinocytes at the wound edges proliferate and phenotype is converted from stationary to motile. This part of the wound healing process is referred to as the re-epithelialization step. It is essential to cover the wound and protect it from infectious agents and water loss. In non-healing wounds, this process is impaired and migration-promoting strategies may offer an attractive solution. In this view, polyelectrolytic nanocomplexes (PEC) bioactivated with RGD-peptides have been developed to induce cell migration in keratinocytes. RGD is a small amino acid sequence, present in several ECM proteins, well known for its ability to induce adhesion and migration in cells [1]. The activity of RGD is increased when properly displayed, e.g., by binding to a macromolecular scaffold. We therefore chose to attach the RGD sequence to the backbone of a chitosan derivative. Chitosan and chondroitin sulfate (CS) are both naturally occurring polymers known for their biocompatibility and biodegradability, and are therefore widely being used as biomaterials. Mixing the two polymers results in the self-assembly of stable PECs [2].

Aim: The goal of this project is to induce interaction between keratinocytes and nanocomplexes, resulting in a change in phenotype and promote cell migration and ultimately wound healing.

Methods: The synthesis of the RGD functionalized chitosan derivative was performed in three steps: trimethylation of chitosan through nucleophilic substitution, carboxymethylation and finally grafting of a RGD to the backbone by carbodiimide chemistry. The PECs were prepared using complex coacervation and their size and properties were investigated by photon correlation spectroscopy (PCS). Finally the polymers and the complexes were tested *in vitro* for its biocompatibility by XTT proliferation tests and for its ability to induce adhesion in adhesion tests with immortalized human keratinocytes, HaCaT.

Results: The PECs were shown to be in the size range of 200–300 nm, with some swelling when suspended in physiological medium. The RGD functionalized chitosan derivative was shown to slightly decrease the cell viability in its soluble form, but no toxicity was observed when complexed with CS even at high concentrations. Finally, adhesion tests have shown a dose-dependent cell attachment of HaCaT-cells to coatings of our RGD-functionalized chitosan derivative.

Conclusions: RGD-functionalized nanocomplexes with promising properties for cell adhesion have been developed for the promotion of wound healing.

Keywords: Polyelectrolytic nanocomplexes, RGD, cell adhesion, re-epithelialization.

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P-20

In Vitro Evaluation of the Adhesion Activity of GRGDS-Chitosan Derivate

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Introduction: Wound healing involves the proliferation phase, the inflammatory phase and the remodeling phase [1]. During this repair process, fibroblasts and keratinocytes become migratory and crawl into and over the wound. Dysfunction of the healing might lead to impaired adhesion and migration. Induction of adhesion and migration are interesting therapeutic strategies. The GRGDS peptide as part of fibronectin is an integrin ligand able to promote cell adhesion and migration [2]. The peptide needs to be displayed correctly for optimal receptor binding, e.g., by grafting to a chitosan derivative (CM-TMC) backbone.

Aims: The goal of this project was to evaluate the adhesion activity of the GRGDS-chitosan derivate by *in vitro* tests on human dermal fibroblasts.

Methods: Adhesion tests were performed to determine the ability of GRGDS-functionalized chitosan derivate to induce cell adhesion. Human dermal fibroblasts were used as cell model. Briefly, the poly-

mer was coated by adsorption on the bottom of the well overnight at 4 °C. The well was washed once with water and blocked 1 h with BSA 1%. The cells were added to the wells and incubated for 35 min before being fixed and stained. The level of adhesion was determined by absorbance measurements at 570 nm. First, a dose response curve was done to show bioactivity of the peptide. The specificity of the activity was studied by two methods: (i) Inhibition test using soluble GRGDS peptide as a competitive inhibitor; (ii) Synthesis of SDGRG-CM-TMC as a control (scrambled peptide of identical amino acid composition as the GRGDS-CM-TMC but no activity).

Results: A dose-response curve was obtained showing that the cells attach to the polymer. In the inhibition test, a decrease of the cell adhesion in the wells containing the free peptide was clearly observed. Moreover, the phenotype changed from being spread in the wells without free peptide (characteristic of cell adhesion) to round in the presence of free peptide. This shows that the activity is specific to the GRGDS peptide. Additionally, the results from the scrambled peptide, SDGRG, showed that the bioactivity of the polymer was due to the presence of the GRGDS peptide, since the wells coated with the scrambled peptide showed low or no adhesion in comparison to GRGDS-CM-TMC, and confirming the specificity of the GRGDS bioactivity.

Conclusions: The ability of GRGDS-CM-TMC to induce adhesion in human dermal fibroblasts was shown. The specificity of the adhesion to GRGDS was evidenced through adhesion assays, including inhibition assays in the presence of free GRGDS and assays with the scrambled peptide SDGRG as a control.

Keywords: Cell adhesion, fibroblast, GRGDS, wound healing.

References:
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[2] M.D. Pierschbacher and E. Ruoslahti. Nature 1984; 309: 30–33.

P-21

CYP3A4-Mediated Ketamine N-Demethylation and Its Inhibition *In Vitro* by Enantioselective Capillary Electrophoresis

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Introduction: The metabolism of ketamine, a chiral phencyclidine derivative used as an anesthetic, has been studied in humans and various animal species, both *in vivo* and *in vitro.* The pathway through N-demethylation to norketamine followed by hydroxylation of norketamine involves many cytochrome P450 (CYP) enzymes, which in turn might be inhibited, induced or occupied by co-administrated drugs. Furthermore, a possible stereoselective metabolism of both enantiomers would result in different pharmacokinetic profiles. Finding kinetic parameters to predict potential drug-drug interactions has been of major interest in drug discovery and development.

Aims: The goals of this work were (i) to characterize the complete kinetics of N-demethylation of ketamine to norketamine via CYP3A4 *in vitro* and (ii) to determine the inhibitory effect of ketoconazole, a potent inhibitor of CYP3A4, on this pathway *in vitro,* using enantioselective capillary electrophoresis (CE) with sulfated cyclodextrin as chiral selector.

Methods: Kinetic studies were performed with 10 substrate concentrations of racemic ketamine (5 to 1000 µM) and separately with single enantiomers (2.5 to 500 µM). Inhibition studies at four substrate concentrations (from 0.25 K_m to 2 K_m) were made with inhibitor concentrations ranging between 0.1 and 2 µM. After an incubation time of 8 min, samples were extracted at alkaline pH, redissolved in 50 µL of 5 mM Tris-phosphate buffer (pH 2.5), and analyzed by enantioselective CE at pH 2.5 using sulfated cyclodextrin as chiral selector. The kinetic data were analyzed by two kinetic models (Michaelis-Menten, Hill equation) using nonlinear least square regression analysis. Various inhibition models and model comparisons with F-test ($p = 0.05$) were investigated and kinetic curves were compared with paired t-test. A p-value < 0.05 was considered significant.

Results: Incubation of racemic ketamine with CYP3A4 revealed data which, for both enantiomers, fitted better to the Michaelis-Menten model. K_m and V_{max} for *S*-norketamine formation were higher compared to those determined for *R*-norketamine. Incubation of single enantiomers resulted in higher K_m and V_{max} values which were significantly different and thus suggested a stereoselective CYP3A4 mediated N-demethylation of ketamine. In presence of ketoconazole as inhibitor, data could best be fitted to an one-site competitive model and inhibition constants K_i were calculated using the equation of Cheng and Prusoff [1]. No stereoselective difference was observed, but inhibition constants for incubation of racemic ketamine were found to be larger compared to those obtained with incubation of single ketamine enantiomers.

Conclusions: The stereoselective kinetics of ketamine N-demethylation by CYP3A4 and its inhibition by ketoconazole could be characterized *in vitro* using enantioselective CE. Further studies and also *in vivo* results would be needed to emphasize the clinical relevance of the calculated kinetic parameters K_m, V_{max} and K_i. Nevertheless, possible drug-drug interactions should be considered when racemic drugs are administered *in vivo.*

Keywords: Capillary electrophoresis, ketamine, CYP3A4, kinetics, inhibition constant.

Reference:
[1] Y. Cheng, W.H. Prusoff. Biochem Pharmacol 1973; 22: 3099–3108.

P-22

Study of the Properties of a Polyurethane Polymer (PU(TEG-HMDI)) as an Excipient for Controlled Drug Delivery

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Introduction: Polyurethanes (PUs) are materials widely used in industry. They are mostly, but not only, prepared by step-growth polymerization between diisocyanates and diols or polyols. Their use in biomedicine is being widely investigated due to their low toxicity, excellent biocompatibility, potential biodegradability, and low thrombogenicity. Although most of their biomedical applications are as long-lasting thermoplastic elastomers, they can also be used as non-permanent medical devices. The rational design of polymers tailored to exert distinct biological functions plays an important role in the development of controlled drug delivery systems [1]. Thus, the controlled release of therapeutic molecules can be achieved by anchoring the active agent to polymer structures by means of physical interactions or by covalent linkages.

Aims: To study the ability of a new biodegradable polyurethane polymer (PU(TEG-HMDI)) to act as matrix forming polymer for controlled release tablets.

Methods: Tablets of 250 mg containing PU(TEG-HMDI) and theophylline as model drug have been prepared by direct compression (Bonals A-300). A Sotax HT1 durometer has been used to measure their crushing strength, whereas the *in vitro* release study has been carried out in a Sotax AT7 apparatus using the paddle method with 900 mL of distilled water.
Results: It has been observed that the mixtures prepared with the PU(TEG-HMDI) have adequate mechanical properties, leading to the preparation of tablets with very high crushing strength (above 160 N in some cases). The results of the dissolution assay show that for matrices containing 20% of polymer, only 52% of theophylline has been released after 8 h. Furthermore, a remarkably low polymer fraction as is 10% w/w has been enough to control the drug release for 8 h.
Conclusions: It can be concluded that the new biodegradable polyurethane polymer (PU(TEG-HMDI)) shows adequate compactibility as well as a high ability to control the drug release.

Keywords: Matrix tablets, theophylline, biodegradable polymer, controlled release.

Reference:
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P-23

Influence of the Drug Particle Size on the Release Behaviour of Paracetamol-MCC Pellets

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Introduction: The percolation theory is a statistical theory to study disordered or chaotic systems. It was firstly applied to the pharmaceutical field in 1987 and since then, the application of its concepts has allowed to explain the release behaviour of numerous pharmaceutical formulations including inert and hydrophilic matrix tablets, and more recently clozapine matrix pellets [1–3].
Aims: The aim of this study is to investigate the influence of the drug particle size on the release behaviour of paracetamol and microcristalline cellulose (MCC) pellets, applying the percolation theory.
Methods: Two batches of pellets containing 20% of two different granulometric fractions of paracetamol and 80% of MCC (vivapur 101) were prepared by extrusion (Pharmex 35T Gabler Maschinenbau)-spheronisation (Sphaeromat SPH 250 MA Gabler Maschinenbau). Release studies were performed using the paddle method in a USP dissolution apparatus (Sotax AT7) with 900 ml of distilled water.
Results: It has been observed that the paracetamol particle size has an influence on the release rate from matrix pellets. The batch with the bigger paracetamol particle size shows a faster release rate at the beginning, due to the higher diameter of the pores obtained. Nevertheless, in a later phase the release rate of both batches becomes similar. This fact can be explained according to the percolation theory, since a smaller drug particle size is related to a more extended percolating cluster of this component (the drug in this case), leading to a higher efficiency in the drug release.
Conclusions: The size and distribution of the paracetamol particles exert a clear influence on the release behaviour of the equally sized pellets obtained by extrusion-spheronisation. This influence governs the initial burst effect and the obtained release profiles. The percolation theory provides a rational explanation for this influence.

Keywords: Pellets, paracetamol, MCC, percolation theory, particle size, release rate.

References:
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[3] A. Aguilar-de-Leyva et al. Int J Pharm 2011; 404: 133–141.

P-24

Influence of Tartaric Acid Pellets on the Release Behaviour of a Basic Drug

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Introduction: The solubility of a drug is a very important factor influencing its gastrointestinal absorption from oral dosage forms. Papaverine is a weak base with pH-dependent solubility that has a poor solubility at pH values higher than 6. Organic acids can be added to the formulations acting as pH modifiers contributing to the ionization of basic drugs and thus enhancing its solubility. Pellets formulations have an increasing demand in the pharmaceutical market. A new strategy to solve bioavailability problems consist of making coated pellets using a functional core instead of an inert core. In this sense, initial cores made of citric acid, or more recently tartaric acid, are appearing in the market. This way, an important step of the coating process can be avoided, reducing the process cost and time. Nevertheless, it is important to verify that a possible leaching of these soluble acids is not compromising their performance.
Aims: The objective of this study is to investigate the influence of an acid microenvironment obtained with tartaric acid core pellets on the release profiles of papaverine HCl.
Methods: Pellets containing papaverine HCl have been prepared using both standard sugar cores and tartaric acid cores (Pharmatrans Sanaq) in a fluid bed system (Innojet type Ventilus-1) with opadry®, HPMC and an ethylcellulose based suspension (Surelease®). Release studies have been performed using the basket method in a dissolution apparatus (Sotax AT7 smart) with 900 mL of pH 6.8 medium.
Results: While the percentage of papaverine HCl released from pellets with sugar cores is lower than 30% after 8 h, pellets containing tartaric acid cores released 100% of papaverine HCl after 3 h.
Conclusions: The low solubility of papaverine HCl at pH 6.8 makes difficult the development of a controlled release dosage form. It has been demonstrated that this drawback can be overcome using tartaric acid cores for the manufacturing of pellets. This way the entire dose is released and the kinetic of the process can be modulated by the formulator.

Keywords: Papaverine HCl, tartaric acid cores, functional starter pellets, drug solubility.

P-25

Analysis of the Utilization of Somatic Medication by Schizophrenic Patients

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Introduction: Schizophrenia is a mental disorder characterized by a disintegration of thought processes and emotional responsiveness that affects around 1% of the population. Schizophrenic patients are frequently polymedicated since in addition to antipsychotic polypharmacy, which is frequent in clinical psychiatry, they are commonly prescribed other CNS drugs. Furthermore, high prevalence of somatic illnesses such as diabetes, cardiovascular risk factors or respiratory diseases have been reported among these patients, so somatic medication is frequently added to the treatment of the patient. The Andalusian Public Foundation for the Social Integration of the People with Mental Disorders (FAISEM) offers a program which provides residence to schizophrenic patients who cannot live with their relatives as a result of their sickness.
Aims: The aim of this study is to analyze the utilization of somatic medication more frequently prescribed in two groups of schizophrenic patients of FAISEM in Seville (Spain) and to determine the presence of polypharmacy.
Methods: Study of the pharmacotherapeutic history of every schizophrenic patient of "Sheltered Homes" (SH1) and "Supervised Housing" (SH2) of FAISEM and descriptive analysis of the utilization of somatic medication and polypharmacy using the SPSS software version 17.0.
Results: Antipeptic ulcer drugs are the group of somatic medication more frequently used, being employed by 37.35% and 25.68% of the patients of SH1 and SH2, respectively. They are followed by antihypertensive and hypolipemic drugs. The proportion of polymedicated patients (patients that receive three or more medicaments a day) is 93.98% and 82.43% in SH1 and SH2, respectively.
Conclusions: The majority of the schizophrenic patients studied are polymedicated. Polypharmacy represents an important risk for the patient's health as a consequence of the higher probability of appearance of drug interactions and side effects.

Keywords: Schizophrenic patients, somatic medication, polypharmacy.

P-26

Isothermal Titration Calorimetry – Insights into the Driving Force of Molecular Interaction

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Introduction: The animal lectin myelin-associated glycoprotein (MAG), which is a member of the Siglec family (sialc acid-binding immunoglobulin-like lectin), inhibits the regrowth of nerve roots in the central nervous system (CNS) after injury. A neuraminic acid derivative with nanomolar affinity towards MAG and the therapeutic potential for the treatment of parapeglia was previously identified [1].
Aims: To gain insights into the driving force controlling the binding of the sialic acid derivative to MAG.
Methods: Isothermal titration calorimetry (ITC) provides a method to dissect the standard free energy of binding (ΔG°) into the standard enthalpy of binding (ΔH°) and the standard entropy of binding (ΔS°). The enthalpic term gives a direct readout of the contributions from formation/removal of non-covalent interactions. The entropic term gives information about the order of the system [2].
Results: ITC can be used to get further information about the driving forces of a biomolecular interaction were heat is absorbed or released upon binding. The binding of MAG antagonist is mainly enthalpically driven with negligible entropic contributions.
Conclusions: With ITC fundamental information of a binding event can be obtained. It exhibits valuable information regarding further steps of lead optimization.

Keywords: ITC, Siglec, MAG.

References:
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P-27

TLR and NLR Agonist Functionalized Chitosan Nanoparticles

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Introduction: To develop a preventive DNA vaccine against *M. tuberculosis* and for recognition of the bacteria, agonists of Toll-like receptors (TLR) and cytosolic NOD-like receptors (NLR) have been considered. TLR and NLR are able to elicit a significant innate immune response which, in turn, affects strongly the initiation of adaptive immunity.
Aims: The objective of this study is to prepare and characterize nanocarrier systems decorated with TLR-9 and NLR-2 receptor targeting moieties. The aim was to produce nanoparticles of 200–600 nm mean diameter and a positive zeta potential. Plasmid DNA containing an unmethylated CpG sequence is adsorbed to the particle surface to target TLR-9, expressed in the endosomes. Further, muramyl dipeptide, a ligand of NOD-2 was incorporated into the particles, maintaining the positive charge of the particle surface. After having prepared the nanoparticles the loading of pDNA and MDP was determined.
Methods: Chitosan was used to synthesize the cationic derivative N,N,N-trimethylchitosan (TMC) and characterized via NMR. The nanoparticles were formed by complex coacervation employing two oppositely charged polymers. MDP was incorporated into the nanoparticles and incubated in a pDNA solution to adsorb the plasmid on the outer surface. The size and zeta potential of plain, MDP loaded, pDNA loaded, and MDP + pDNA loaded nanoparticles were measured by photon correlation spectroscopy and laser doppler anemometry, respectively, using a zetasizer. The loading level of MDP and pDNA was determined by assaying the supernatant after having prepared the loaded particles with spectrophotometric and chromatographic methods.
Results: The cationic polymer TMC was successfully synthesized with a degree of trimethylation of 31%. Using chondroitin sulfate as an anionic agent nanoparticles with a size below 600 nm were formed. Their potential as carrier for a NOD-2 ligand has been proven by incorporating muramyl dipeptide (MDP), maintaining the positive charge of the particle surface. Plasmid DNA has been successfully adsorbed on the outer surface, keeping the size of the nanoparticles within the required limits. Methods to quantify the muramyl dipeptide and pDNA were developed.
Conclusions: This study demonstrated that nanoparticles decorated with TLR and NLR agonists can be prepared by a complex coacervation method. The polydispersity of the particles was low and a high adsorption efficiency of pDNA was achieved. In conclusion, this new nanoparticulate system offers an interesting poten-

tial as a carrier for ligands to induce and enhance a cellular immune response.

Keywords: DNA vaccine, chitosan nanoparticles, Toll-like receptor, Nod-like receptor.

P-28

LESA-DMS-MS Combined: Screening of Cocaine and Isomeric Metabolites from Tissue Sections

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Introduction: Direct analysis of illicit drugs is attractive for forensic investigations in postmortem human tissue sections since it requires minimal or no sample preparation. However, without chromatographic separation beforehand, the main issues remain isobaric interferences due to the complexity of tissue samples and analysis of isomeric compounds, which is not allowed. To overcome these issues, liquid extraction surface analysis (LESA) is combined herein with differential ion mobility spectrometry hyphenated to mass spectrometry (DMS-MS), a gas-phase separation of ions at atmospheric pressure, based on the difference of ionic mobility at low and high electrical field.
Aims: Detection of cocaine directly from tissue sections with minimal sample preparation and subsequent gas-phase separation of isomeric metabolites (benzoylecgonine, BZE; norcocaine, NCOC) to increase selectivity.
Methods: Two kidney samples were provided by the Institute of Legal Medicine of Zurich and referred as "positive sample" (drugs detected in kidney dialysate by immunoassays IA) and "negative sample" (no drugs detected by IA). Frozen samples were brought up to –20 °C, cut at 12 µm thickness and immediately transferred onto a stainless steel plate. Surface sampling was performed on tissue sections and the extracts were subsequently infused into the mass spectrometer using a chip-based nanoelectrospray (nES) device. A 0.3 psi gas pressure and a 1.7 kV voltage were applied to the sample to generate the nES. MS and MS/MS acquisitions were performed in positive ionization polarity on a Q TRAP 5500 mass spectrometer equipped with a DMS device in front of the orifice plate. Acquisitions were performed in information dependant acquisition (IDA) mode. The survey MS experiment was performed by ramping the compensation voltage from – 40 V to 0 V in the selected reaction monitoring (SRM) mode. Dependant scans were based on both MS/MS and MS3 experiments for confirming the compounds identity.
Results: *Evaluation of different DMS modifiers for the separation of cocaine metabolites isomers.* It has been demonstrated that the addition of an organic modifier such as acetone, acetonitrile or toluene in the gas phase considerably enhances the separation of isomeric BZE and NCOC by the dynamic formation of clusters between the modifier and the ions. Their difference in compensation voltage values is 8.7 V with acetone for a baseline resolution. *Investigation in tissue sections.* The SRM traces for liquid extracts from tissue samples confirm the presence of both BZE and NCOC in the positive sample with sufficient selectivity. As expected, no drugs could be detected in the negative sample. The structural confirmation in both MS/MS and MS^3 experiments is given by the following specific ions for each metabolite: *m/z* 82 and *m/z* 150 (water loss from *m/z* 168 fragment) for BZE; *m/z* 68 and *m/z* 136 (loss of $O{=}CH_2$ from *m/z* 168) for NCOC.
Conclusions: The results given by LESA-DMS-MS are in accordance with previous IA experiments performed routinely at the Institute of Legal Medicine. LESA and DMS combined provide new perspectives for the direct analysis of illicit drugs and metabolite isomers from tissue sections without prior chromatographic separation, increasing assay selectivity compared to other direct analysis techniques such as MALDI-MS. Finally, information dependant acquisition mode combining SRM, MS/MS and MS^3 experiments is beneficial for the simultaneous screening and relative quantitation of illicit drugs in tissue sections.

Keywords: Surface sampling, differential ion mobility spectrometry, tissue analysis, toxicological screening, structural isomer.

P-29

Innovative Fluid Bed Pelletizing Technologies for Taste Masked Micropellets Design

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Introduction: The oral administration of drugs exhibiting poor organoleptic qualities through solid dosage forms requires an acceptable degree of palatability, enhanced performance and acceptability. In the pharmaceutical industry, the need for improved patient compliance has prompted the development of taste masking techniques to prevent active pharmaceutical ingredients exposing an unpleasant taste, aftertaste or smell. In the recent years, enormous progress in formulation design and development has given rise to more sophisticated novel oral drug delivery systems in which the role of micropellets is increasing. For this purpose, pelletization technologies provide ideal cores for application of functional coatings and to mask inconvenient taste, and therefore, offer a great scope for innovations in taste masking to reduce and inhibit oral pharmaceuticals' bitterness.
Aims: This work presents innovative strategies for the taste masking of micropellets using different fluid bed pelletizing technologies and indicates the most interesting features of each approach.
Methods: The development of taste masked micropellets systems comprising highly bitter active pharmaceutical ingredients was achieved using conventional and innovative fluid bed technologies. The study investigates different manufacturing strategies for taste masking purposes using conventional fluid bed bottom spray coating (Wurster mode), as well as innovative continuous fluid bed spray granulation and layering process (MicroPellet delivery system, MicroPx™) and direct modified rotor fluid bed spheronization (Direct Pelletizing System, CPS Technology™). Micropellets were evaluated in terms of particle size, morphology (Scanning Electron Microscopy), taste masking and *in vitro* dissolution performance.
Results: The study demonstrates that taste masked micropellets of highly bitter drugs were successfully achieved. Fluid bed pelletizing technologies intended for taste masking cover a very broad range of micropellets features including high drug loading, small particle size, narrow particle size distribution, smooth and uniform surface, as well as manufacturing strategies.
Conclusions: The methods most commonly involved for achieving taste masking often fail in novel oral drug delivery systems. More sophisticated strategies using micro pelletization technologies, associated with a Wurster coating process, offer a solution for effective taste masking of highly bad tasting drug substances.

Keywords: Taste masking, technology, micropellets, design.

P-30

In Vivo Imaging of Oral Enzyme Activity in the Gastrointestinal Tract – Application to Celiac Disease

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Introduction: Therapeutic enzymes are administered orally to treat several diseases, such as pancreatic insufficiency and lactose intolerance [1]. Celiac disease (CD) is a highly prevalent (0.5–1%) immunogenetic enteropathy induced by ingestion of wheat proteins (*i.e.,* gluten) which is currently treated by lifelong elimination of gluten from the diet [2]. Oral administration of proline-specific endopeptidases (PEPs) is a potential adjuvant therapy for CD because these enzymes can efficiently digest gluten peptides and thus minimize their toxicity. Due to the proteinaceous nature of PEPs, oral enzyme activity may be impaired by means of pH changes and degradation by digestive enzymes in the gastrointestinal (GI) environment.
Aims: In the present work a convenient fluorescence-based assay to monitor the activity of exogenous enzymes in real time *in vivo* in the GI tract was developed using PEPs as model application [3]. This study incorporated PEPs from *Flavobacterium meningosepticum* (FM), *Myxococcus xanthus* (MX), and *Sphingomonas capsulate* (SC).
Methods: *In vitro* stability of PEPs was evaluated by incubating individual enzymes in simulated gastric or intestinal fluid USP, and at pH 4.5 with pepsin. Residual activity was analyzed using the dipeptic model substrate Z-Gly-Pro-*p*NA. For *in vivo* imaging experiments the PEP-specific gluten peptide LPYPQP was labelled with a fluorescent dye (λ_{em} = 700 nm) and corresponding quencher. Upon cleavage by PEPs, fluorescence emission was detected and analyzed using an *in vivo* imaging system. Peptide and enzymes were orally administered to rats and the impact of modification of the GI environment on PEPs' activity was examined.
Results: The assay revealed distinct differences between PEPs' performance *in vitro* and *in vivo. In vitro,* enzymes were rapidly inactivated at low pH and in the presence of GI proteases. In rats however, FM PEP showed significant gastric activity compared to MX PEP. Coadministration of an antacid drug (*i.e.,* magaldrate) significantly ameliorated gastric activity of MX PEP due to increased pH and/or inhibition of stomach proteases. In the small intestine, comparable fluorescence signals were observed for both PEPs. Even though MX PEP was not active under gastric conditions, it could at least partially recover its activity in the small intestine suggesting potential regeneration of enzyme conformation.
Conclusions: This simple *in vivo* test can be useful to identify differences between PEPs which cannot be easily predicted from *in vitro* studies. The assay constitutes an important and timely method for understanding and optimizing key factors affecting therapeutic efficiency of orally administered enzymes.

Keywords: Oral enzymes, fluorescence imaging, celiac disease, proline-specific endopeptidases.

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P-31

Migraine in Patients with Rosacea

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Introduction: Rosacea is a common chronic skin disease. Abnormalities of small blood vessels are considered as key causative factors, but various hypotheses have been postulated. Like rosacea, migraine has been associated with abnormal vascular physiology. Triptans (selective $5HT_1$-receptor agonists) and ergot alkaloids stimulate cranial vasoconstriction and are effective against migraine headache. An increased risk for migraine patients to develop an incident rosacea diagnosis has been postulated. However, only few studies explored this association and the results remain controversial.
Aims: This study aimed to analyze the association between migraine and the risk of developing rosacea. We further explored a possible association between use of triptans and ergot alkaloids and incident rosacea.
Methods: We conducted a case-control analysis using the UK-based General Practice Research Database. We included 60,042 rosacea patients and the same number of controls. We identified cases with an incident diagnosis of rosacea between 1995 and 2009, and matched each control to a case patient on age, gender, general practice, and index date. We compared the prevalence of migraine and the exposure to triptans and ergot alkaloids prior to the index date between cases and controls.
Results: A history of migraine was slightly more prevalent in cases with rosacea as compared to rosacea-free patients (odds ratio, OR 1.23, 95% CI 1.17–1.28), after adjusting for smoking, alcohol consumption, body mass index, diabetes mellitus, hypertension, and use of calcium channel blockers. The highest OR associated with migraine was found in women above the age of 50 years (adj. OR 1.41, 95% CI 1.29–1.53). Adjusting for use of hormone replacement therapy did not substantially influence the OR (adj. OR 1.32, 95% CI 1.22–1.44). The ORs were similar for patients who used triptans (adj. OR 1.30, 95% CI 1.19–1.41) and for patients not using triptans (adj. OR 1.20, 95% CI 1.14–1.26), as compared to patients without migraine. Migraine treatment with ergot alkaloids did also not reveal a significant difference in ORs. Stratification by the number of prescriptions of triptans and ergot alkaloids, as an indicator for migraine severity, did not change ORs.
Conclusions: Our findings suggest that patients with migraine, with or without triptan use, may be at a marginally increased risk for an incident rosacea diagnosis. The frequency of triptan and ergot alkaloid use, a marker for migraine severity, did not influence the risk estimate. Women of 50 years or older were at a slightly higher risk of developing rosacea.

Keywords: Rosacea, migraine.

P-32

Dual Inhibition of the Serotonin and Norepinephrine Transporter Prevents Acute Effects of MDMA

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Introduction: ±3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) induces non-vesicular release of serotonin (5 HT), norepinephrine (NE), and dopamine (DA) through an interaction with the corresponding monoamine transporter. While DA is generally thought to mediate the reinforcing effects of drugs of abuse, 5-HT and NE release may be responsible for the acute subjective response to psychostimulants.
Aims: The aim of the study was to assess the role of the NE and 5-HT transporter in the acute effects of MDMA in humans.
Methods: We investigated the pharmacodynamic and pharmacokinetic interactive effects of duloxetine and MDMA in 16 healthy human subjects using a double-blind, placebo-controlled crossover design.
Results: Duloxetine pretreatment prevented MDMA effects including elevations in circulating NE, increases in blood pressure and heart rate, as well as subjective well-being, drug high, drug liking, extroversion, and feelings of closeness to others. The pharmacodynamic response to MDMA was blocked by duloxetine despite duloxetine-associated elevations in plasma levels of MDMA.
Conclusions: The findings indicate that the psychotropic effects of MDMA in humans depend primarily on transporter-mediated release of both 5-HT and NE. 5-HT and NE transporters may be targets for treatment of psychostimulant dependence.

Keywords: 3,4-Methylenedioxymethamphetamine, serotonin transporter, norepinephrine transporter, pharmacokinetics, pharmacodynamics.

P-33

A Quantitative LC-Tandem Mass Spectrometry Method for the Analysis of Thiol Peptides in Kinetoplastids

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Introduction: Kinetoplastids, a group of unicellular flagellate protozoa including *Trypanosoma* and *Leishmania,* possess a unique thiol redox metabolism which unlike the glutathione/glutathione reductase system in mammals uses the trypanothione/trypanothione reductase system. The synthesis and reduction of trypanothione are essential for the maintenance of their reducing intracellular environment and their sensivity to oxidative stress. The absence of trypanothione in mammals renders trypanothione metabolism an attractive drug target.
Aims: To develop and validate an analytical method for the quantification of both trypanothione (TS_2, TSH_2) and its precursor glutathione (GSH) from *Trypanosoma brucei rhodesiense.*
Methods: To extract major thiols compounds from trypanosomes, a simple four step protocol was developed by pelleting, washing, cell lysing, and concentrating. Using a Waters UPLC-DAD coupled to a triple quadrupole detector (MS/MS), extracted and enriched samples were separated on a C18 reversed phase column and detected in positive ElectroSpray Ionization (ESI+) mode with multiple reaction monitoring (MRM). Linear calibrations curves were performed over the concentration range of 10–5000 ng/mL and were validated according FDA Bioanalytical Guidelines.
Results: The method showed lower limits of quantification (LLOQ) of 10 ng/mL for GSH, 25 ng/mL for TS_2, and 50 ng/mL for TSH_2. These following MRM transitions were selected for quantitation of thiolpeptides: GSH 308.4 > 179.2, TS_2 361.5 > 231.3, and TSH_2 361.9 > 233.3. Preliminary results showed that *T. brucei rhodesiense* contains 4.6 ± 1.2 µM GSH and 2.4 ± 0.4 µM of total trypanothione (n = 3 ± SEM).
Conclusions: This is the first simple study, which uses a LC-MS/MS method to quantify major thiol peptides in trypanosomes. It will be used to determine the effects of experimental bioactive compounds on enzymes involved in thiol biosynthesis and homeostasis.

Keywords: UPLC-MS/MS, trypanothione, glutathione, *T. brucei rhodesiense*.

P-34

Using Microemulsions to Increase Cutaneous Delivery of Clotrimazole

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Introduction: Azole antifungals are a first-line treatment for skin mycoses which, although not life-threatening, are extremely prevalent, affecting up to one quarter of the world's population. Clotrimazole (CMZ) was one of the first azole antifungal drugs developed and is widely used. However, its lipophilicity and low water solubility make its formulation difficult – this can result in poor cutaneous bioavailability and so reduce efficacy. Although microemulsion formulations have been previously shown to increase topical delivery of poorly soluble lipophilic compounds, to-date there are no reports on their use to improve delivery of CMZ.
Aims: To develop a microemulsion-based formulation for CMZ, to quantify drug delivery across porcine skin and to evaluate antifungal activity *in vitro.*
Methods: Microemulsion components were chosen after determination of the saturation solubility of CMZ in a series of solubilizers/cosurfactants, oils and surfactants that have been approved for topical application. Microemulsion composition was optimized using ternary/pseudoternary phase diagrams. CMZ microemulsions (1% and 2% w/w) were formulated by simple mixing and subsequently characterized. Skin transport of CMZ from the microemulsions and from two marketed formulations (Canesten®, 1% w/w, and Gyno-Canesten®, 2% w/w) was studied using conventional Franz diffusion cells (A = 1.9 ± 0.1 cm^2) and full thickness porcine ear skin. An in-house HPLC method was used to quantify CMZ in the saturation solubility and skin transport experiments. The optimized microemulsions were tested for their antifungal activity against *Candida albicans* ATCC 10231.
Results: The best solubilizers/cosurfactants, oils and surfactant for CMZ were, respectively, Transcutol P (88.5 mg/ml), Capryol 90 and Lauroglycol 90 (83.8 and 28.2 mg/ml, respectively) and Labrasol (18.8 mg/ml). Ternary/pseudoternary phase diagrams were prepared for the different oil, surfactant/cosurfactant combinations. Thermodynamically stable CMZ microemulsions (1% and 2% w/w) were formulated and characterized with respect to globule size and pH. The optimized CMZ microemulsions (1% and 2% w/w) exhibited globule sizes of 160 and 196 nm, respectively. The pH of each microemulsion was also suitable for topical application (6.1 and 6.35, respectively). Although no CMZ was detected in the receiver compartment after 6 h permeation from any of the formula-

tions studied, there was significant CMZ deposition in the skin. The amount of CMZ retained in the skin from the optimized 1% w/w microemulsion was almost 6-fold higher than that from Canesten® (7.0 ± 1.6 and 1.2 ± 0.5 µg/cm^2, respectively). Similarly, the optimized 2% w/w microemulsion resulted in a 2.5-fold improvement in CMZ deposition as compared to that seen with Gyno-Canesten® (13.2 ± 3.7 and 5.4 ± 1.4 µg/cm^2, respectively). Furthermore, the optimized 1% w/w microemulsion also demonstrated a higher activity against *Candida albicans* than Canesten® (the diameters of inhibition were 6.1 and 4.5 cm, respectively)

Conclusions: Thermodynamically stable CMZ microemulsions were successfully prepared using approved excipients. Skin deposition of CMZ using these microemulsions was significantly higher than that observed with marketed formulations. Finally, microemulsions also showed a higher antifungal activity than the marketed formulation against *Candida albicans*.

Keywords: Clotrimazole, skin delivery, microemulsion, antifungal activity.

P-35

Comparison of a LC-MS and a GC-MS Method with Internal Standards to Analyze Phenytoin from Different Biological Samples

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Introduction: Phenytoin (PHT) is used to treat and/or prevent seizures in neurosurgical patients. Little is known about the fate of PHT in the brain (target organ) and the correlating concentrations in other biological samples commonly used for TDM, like plasma. To investigate this pharmacokinetic correlation, a sensitive, reliable, and cost-effective analytical method is needed.

Aims: The key objective of the study is to compare and evaluate a LC-MS versus a GC-MS method to analyze PHT in different biological matrices.

Method: A GC-MS and a LC-MS method for PHT analysis in biological samples were established, and their characteristics compared using samples of *ex vivo* microdialysis, artificial cerebrospinal fluid (aCSF), blood, and saliva. Calibration range of PHT covered the expected concentrations in patients. The GC-MS analysis required a solid-phase extraction (SPE) for clean-up of the samples (≥ 50 µL). Derivatization of the residue was made with trimethylsulphonium hydroxide. 5-(p-Methylphenyl)-5-phenylhydantoin (MPPH) was used as internal standard (I.S.). The calibration curve ranged from 50 to 1200 ng. For LC-MS, d_{10}-PHT (100 µg/mL in MeOH) was used as I.S., diluted to 50 ng/mL with $HClO_4$ for deproteinization. 75 µL of this solution was added to 25 µL sample. The calibration curve ranged from 10 to 2000 ng. Both methods were validated according to ISO/FDA Guidance for Industry. Selectivity, sensitivity, recovery, limit of detection (LOD), limit of quantification (LOQ), accuracy, linearity of the curves, and extract stability for both procedures were assessed. For performance assessment, the sample volume needed, the duration of an analysis, and the costs of materials were considered.

Results: *GC-MS.* Selectivity and sensitivity were checked; all blank samples were negative. Recovery: Quality control (QC) 100 ng, QC 1000 ng ≤ 8% min/max deviation from target value. LOD = 15 ng, LOQ = 50 ng in all matrices. Accuracy: 1–10% for calibrator (cal)2 (150 ng) to cal6 (1200 ng) and 20% for cal1 (50 ng). The calibration curve was linear (r^2≥0.995, aCSF n=8, blood n=2, and saliva n=2). Dried extracts were stable > 4 weeks (min/max deviation 4%). Reinjection and storage (33 h) on the autosampler showed no effect. The run time was 30 min per analysis; the clean-up time for 25 samples took 5 h.

LC-MS. Selectivity and sensitivity: blank samples were negative. Recovery: min/max deviations from target value were 10% for QC 10 ng and 3% for QC 1600 ng. LOD <<10 ng, LOQ = 10 ng. Accuracy: 1–8% for cal2 (20 ng) to cal8 (2000 ng) and 3% for cal1 (10 ng). The calibration curve was linear (r^2 ≥0.997, aCSF n= 6, blood n=3, and saliva n=3). Reinjection after 7 days: no difference in accuracy was detectable. The run time was 7 min per analysis, preparation time for 182 samples took 6 h.

Conclusions: The LC-MS method showed better performance. The smaller sample volume, the easier sample preparation, and the fewer chemicals needed made the LC-MS method preferable. Furthermore, the LC-MS is cheaper, less time-consuming, and the linearity of the calibration covers a larger range (10–2000 ng), exceeding by far the expected PHT concentrations in biological samples. Thus, especially for larger sample numbers as in pharmacokinetic studies, the LC-MS method shows great advantages over GC-MS.

Keywords: Phenytoin, GC-MS, LC-MS, biological samples.

P-36

Identification of $GABA_A$ Receptor Modulators with a Twist: Discovery of Aristolactone in a Commercial Sample of *Bupleurum chinense* Root

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Introduction: γ-Aminobutyric acid type A ($GABA_A$) receptors are the major mediators of fast synaptic inhibition in the central nervous system. In a screening of a plant extract library for $GABA_A$ receptor modulatory activity, a petroleum ether extract of a commercial sample of the traditional Chinese herbal drug *Chaihu* (*Bupleurum chinense* DC. root) showed significant activity. The germacranolide identified as responsible for the activity of the extract, however, is a chemotaxonomic marker of the genus *Aristolochia* (Aristolochiaceae). This suggested possible adulteration of the commercial sample. *Aristolochia* species are used in Traditional Chinese Medicine (TCM) but contain highly nephrotoxic aristolochic acids. Their use is no longer permitted in Europe for safety reasons.

Aims: In the present work, we aimed to identify the compound responsible for the $GABA_A$ modulatory activity of the extract. Furthermore, the adulteration of the commercial *Bupleurum chinense* sample with material from an *Aristolochia* species needed to be confirmed with analytical means.

Methods: $GABA_A$ receptor modulatory activity was tested in a two–microelectrode voltage clamp assay using *Xenopus laevis* oocytes transiently expressing the $GABA_A$ receptor subtype $\alpha_1\beta_2\gamma_{2s}$. HPLC–based activity profiling [1] combined with high resolution LC–MS and microprobe NMR was used for isolation and structure elucidation of the active compound. The presence of an *Aristolochia* species in the commercial sample was confirmed using a validated HPTLC protocol for the detection of aristolochic acids [2], and corroborated by macroscopic and microscopic examination of the drug.

Results: The germacranolide aristolactone was identified as one of the main active compounds of the extract (EC_{50} 56.02 ± 5.09 µM). The herbal sample was confirmed to be a mixture of *Aristolochia manshuriensis* root and *Bupleurum chinense* root.

Conclusions: Aristolactone is a low–potency $GABA_A$ receptor modulator, representing a scaffold previously not reported for this tar-

get. The commercial sample of the Chinese herbal drug *Chaihu* was shown to be adulterated with roots of the nephrotoxic herb *A. manshuriensis*. This case raises concerns about adequate quality control of TCM drugs commercialized in Europe.

Keywords: *Bupleurum chinense, Aristolochia* sp., $GABA_A$ receptor, adulteration, HPTLC.

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P-37

Antitrypanosomal Sesquiterpene Lactones from *Saussurea Costus*

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Introduction: The roots of *Saussurea costus* (Falc.) Lipsch. (Asteraceae) have been used for thousands of years as medicines, incenses and ointments by many cultures in the Himalayas, India, and China. A broad spectrum of biological activities from antiinflammatory to antimicrobial properties have been reported from this species and are commonly related to the presence of sesquiterpene lactones [1]. In an antiprotozoal screening of our 1800 library extracts, the ethyl acetate extract of *S. costus* roots inhibited *Trypanosoma brucei rhodensiense* by 96% at 4.8 µg/mL.
Aims: Isolation and identification of the antitrypanosomal compounds from *S. costus.*
Method: HPLC-based acitivity profiling of 350 µg of active *S. costus* extract was used to determine the active principles [2]. Then these compounds were isolated by HPLC in a semi-preparative scale. Identification of pure compounds was carried out based on 1D and 2D NMR and HRMS. The isolated compounds were tested *in vitro* against *T.b. rhodensiense* for antiplasmodial activity and against rat myoblast L6 cells for cytotoxicity along with two other sesquiterpene lactones, each isolated from the aerial parts of *Laurus nobilis* L. and the leaves of *Eupatorium cannabinum* L., and one commercial compound.
Results and conclusions: The seven isolated sesquiterpenelactones arbusculin B, α-cyclocostunolide, costunolide, parthenolide, eupatoriopicrin, dehydrocostuslactone, and zaluzanine D showed *in vitro* activity against *T.b. rhodensiense* with IC_{50} of 12, 21.9, 1.3, 4.4, 0.8, 11.2, and 1.2 µM, respectively. The cytotoxicity in order were 6.2, 19.4, 7.7, 8.3, 5.2, 15.6, and 1.6 µM. The selectivity indices were ranged from 0.5 to 6. The sesquiterpene of the germacranolide class displayed the highest activity, followed by the guaianolides, and eudesmanolides.

Keywords: *Saussurea costus,* Asteraceae, sesquiterpene lactones, antitrypanosomal activity, HPLC-based activity profiling.

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P-38

In Vitro Screening of Traditional South African Malaria Remedies Against *Trypanosoma brucei rhodesiense, Trypanosoma cruzi, Leishmania donovani and Plasmodium falciparum*

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Introduction: Half a billion people get infected with malaria every year, and 1–2 million of them die of the disease [1]. Up to 30 million people annually contract one of the so called "neglected tropical diseases", Chagas disease, human African trypanosomiasis or leishmaniasis and 120,000 of them die of these [2]. Here we report on the outcome of a screen which is the starting point in collaboration between the University of Basel, the Swiss Tropical and Public Health Institute, and the South African Council for Scientific and Industrial Research.
Aims: The aim of this study was to select antimalarial remedies from South African folk medicine and evaluate their antiprotozoal potential against not just malaria parasites but also against the parasites that cause the other main protozoal diseases: human African trypanosomiasis, Chagas disease and leishmaniasis.
Methods: We prepared 300 extracts from traditional antimalarial remedies and screened them *in vitro* for activity against *Plasmodium falciparum, Trypanosoma brucei rhodesiense, Trypanosoma cruzi,* and *Leishmania donovani* at concentrations of 9.7 and 1.8 µg/mL. For the 43 extracts which inhibited the growth of one or more parasites to more than 95% at 9.7 µg/mL, the IC_{50} values against all four protozoal parasites and cytotoxic IC_{50}s against rat myoblast L6 cells were determined.
Results: Amongst the most notable results are the activities of *Agathosma apiculata* (IC_{50} of 0.3 µg/mL) against *Plasmodium falciparum* and *Salvia repens* and *Maytenus undata* against *Leishmania donovani* with IC_{50}s of 5.4 µg/mL and 5.6 µg/mL, respectively.
Conclusions: This screening is the starting point for an HPLC-based activity profiling project in antiprotozoal lead discovery. Further results will be reported in due time.

Keywords: South Africa, antiprotozoal, *Trypanosoma brucei rhodesiense, Trypanosoma cruzi, Leishmania donovani and Plasmodium falciparum.*

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P-39

Injectable Organogels to Treat Tumors Through Combined Hyperthermia and Chemotherapy

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Introduction: Metastatic prostate carcinomas are one of the leading causes for cancer-related deaths nowadays. Once bone metastasis takes place, survival is low. While the use of chemo-therapeutic drugs has shown promise, its current use is limited by systemic side-effects. Treatment could be improved by administering the drug locally within the bone. Furthermore, research has shown that

cancerous cells are more damaged by exposure to high temperatures than normal cells and that association with other cytotoxic therapies can be beneficial.
Aims: To develop and characterize an *in situ* forming implant able to treat bone metastases through chemotherapy (with doxorubicin or docetaxel) and magnetically induced hyperthermia [1]. The injectable formulation is an organogel containing a polymer, cellulose acetate (CA), and an organic solvent, DMSO, in which silica-embedded superparamagnetic beads (SSB) are incorporated.
Methods: In order to determine the appropriate CA and SSB concentrations, organogels were characterized based on rheological behavior, injectability through a vertebroplasty needle (11G), as well as heating capacities and specific power loss when exposed to an alternating magnetic field of 3, 6 and 9 mT. The chosen implant formulation was loaded with doxorubicin or docetaxel and an elution assay was carried out at 37 °C in a physiological saline solution (NaCl 0.9% w/v) followed by drug extraction first with a strong electrolyte (KCl 0.3 M in EtOH/H_2O 30:70 v/v) and secondly with DMSO. To establish if drug-SSB interactions could influence drug delivery, elution and extraction assays were also carried out on SSB suspensions and organogels with CA only. Due to poor water solubility of docetaxel, we incorporated 1% (w/v) of polysorbate 80 into the elution media of the preparations loaded with this drug.
Results: We determined that the ideal formulation for our organogels incorporated 20% (w/v) CA and 40% (w/v) SSB. This SSB concentration allowed for the generation of heat on the cytotoxic range (42–45 °C) when exposed to a clinically acceptable magnetic field [2]. The results of this study showed that positively charged drugs like doxorubicin formed strong electrostatic bonds with SSB that delayed drug delivery and only 29% of the loaded drug was released after 3 days. Docetaxel, did not interact with SSB probably due to its absence of charge and lipophilicity. Docetaxel delivery was only limited by diffusion through the polymeric matrix and it was possible to deliver 66% of the loaded drug after 3 days.
Conclusions: The results indicate that the nature of the chemotherapeutic agent loaded into the implant directly influences drug release. We consider that the use of injectable carriers, capable of delivering antiblastic drugs and generating hyperthermia locally, holds a strong potential for solid tumor therapy. From such treatment would greatly benefit patients suffering from bone metastasis who currently have few efficient treatment options.

Keywords: *In situ* forming implants, anticancer agents, hyperthermia, SPIONS, bone metastasis.

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P-40

Antihypertensive Drugs and the Risk of Developing Gout

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Introduction: Gout is a common rheumatic disease in humans which is characterized by elevated serum uric acid levels, deposition of uric acid crystals in the joint and an acute inflammatory arthritis. While current use of diuretics has been linked to an increased risk of developing gout, the role of other antihypertensive drugs remains largely unknown.
Aims: We aimed at studying the association between use of diuretics or other antihypertensive drugs and the risk of developing gout.
Methods: We conducted a case-control study using the UK-based General Practice Research Database (GPRD). We identified cases aged between 18 and 80 years with an incident gout diagnosis between 1995 and 2009 and matched them to one control patient on age, sex, general practice, calendar time, and years of history in the database. Conditional logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (CIs) of developing gout in relation to previous use of diuretics or other antihypertensive drugs such as beta-blockers (BBs), calcium channel blockers (CCBs) angiotensin converting enzyme inhibitors (ACE-Is), or angiotensin II receptor blockers (ARBs), stratified by timing of use and adjusted for potential confounders.
Results: The study encompassed 55,580 cases with a first-time gout diagnosis and the same number of matched controls. As compared to non-users, current users of diuretics were at an increased risk of developing gout (OR 2.29, 95% CI 1.84–2.86). Current use of other antihypertensive drugs such as BBs (OR 1.19, 95% CI 0.95–1.49), CCBs (OR 0.86, 95% CI 0.66–1.13), ACE-Is (OR 1.25, 95% CI 1.08–1.45), or ARBs (OR 1.1, 95% CI 0.55–2.2) was not associated with a materially altered risk of developing gout.
Conclusions: This analysis suggests that patients with current use of diuretics are at increased risk of developing gout, while use of other antihypertensive drug was not related to an essential altered risk.

Keywords: Gout, antihypertensive drugs, diuretics.

P-41

Fluoroscopic Analysis of Protein Aggregates

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Introduction: Over the last decades there has been a huge increase in biopharmaceuticals on the market. Protein aggregation remains one of the major obstacles in the development of new biopharmaceutical drugs. The most common approaches to overcome this obstacle are modification of the protein structure (either by site-directed mutagenesis or by chemical reactions with polymers) and the choice of excipients in the formulation.
Aims: The aim of this study was to test whether tryptophan-PEG (Trp-PEG) has the potential to inhibit the aggregation of a model protein, Hen egg white lysozyme (HEWL) [1].
Methods: Aggregation was induced by heating HEWL samples with and without Trp-PEG and mPEGOH for 2.5 days at 56 °C in an acidic environment (pH 2). Aggregation was then analyzed by fluorescence intensity, fluorescence anisotropy and circular dichroism. HEWL samples which were not exposed to stressors served as control.
Results: The aggregated lysozyme showed clearly a higher intrinsic fluorescence than the non-aggregated species. The addition of Trp-PEG reduced the fluorescence significantly while addition of mPEGOH showed only a marginal reduction of the fluorescence. The aggregated lysozyme exhibited a higher fluorescence anisotropy than the non aggregated lysozyme. Although showing an increase in anisotropy compared to the non-aggregated lysozyme, the intensity of the Trp-PEG group was not as high as the aggregated lysozyme. CD spectra showed no differences between aggregated lysozyme and aggregated lysozyme with PEG excipients. The non-aggregated lysozyme showed a stronger signal than the aggregated groups.
Conclusions: Protein aggregation is frequently accompanied by an elevated fluorescence as fluorescent amino acids buried inside the

protein are exposed to the solvent in the aggregated state. Therefore the reduction in fluorescence by addition of trp-PEG suggests a major reduction in protein aggregation. These results concur with the results from fluorescence anisotropy. The intensity of the anisotropy correlates to loss of mobility of the fluorophores, therefore aggregates give a stronger signal due to loss of mobility because of the increase in mass. The addition of Trp-PEG resulted in a decreased fluorescence anisotropy compared to aggregated protein, therefore again suggesting a decrease in aggregation. The addition of trp-PEG resulted in no difference to aggregated protein in the CD spectra. This strengthens the hypothesis that trp-PEG inhibits aggregation by sterically shielding hydrophobic patches from each other and not through inhibition of protein unfolding. These results show some promise for the use of trp-PEG as an excipient in pharmaceutical protein formulations.

Keywords: Protein aggregation, non-covalent PEGylation, protein fluorescence.

Reference:
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P-42

Bone Metastases Treatment Through *In Situ* Forming Implant-Mediated Hyperthermia and Chemotherapy

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Introduction: Bone metastases might be efficiently treated using intraosseous implants. In this view, we propose novel formulations that, once injected intratumorally, form a solid implant. Poly(methylmethacrylate) cements (PMMA cements) and cellulose acetate organogels are two relevant formulations already use in vertebroplasty and embolization procedures, respectively. They can be loaded with both an anticancer agent and superparamagnetic beads for combining chemotherapy and hyperthermia, the latter being an effective adjuvant in cancer therapy [1].
Aims: To develop cement and organogel formulations carrying superparamagnetic silica beads (SSB) embedding superparamagnetic iron oxide nanoparticles (SPIONs) and anticancer agents (doxorubicin, DOX). The implant can be heated applying an external magnetic field, sensitizing the surrounding tumoral tissues, while releasing the chemotherapeutic agent. By combining hyper-thermia and chemotherapy, a synergetic effect may be reached improving the therapeutic effects of the implant.
Methods: Cement was prepared by mixing poly(methylmethacrylate) and its monomer in presence of an initiator and an activator. Organogels were prepared using cellulose acetate solution in DMSO (20% w/v). For both kind of implants, SSB and DOX loading was 40% (w/v) and 2.5% (w/w), respectively. *In vitro* DOX release was carried out in a saline media (NaCl 0.9%) at 37 °C and the DOX was analyzed using an UV-VIS spectrophotometer at 479 nm. *In vitro* toxicity of the implants was tested using the XTT proliferation kit. Immortalized human prostate cancer cells, PC3, were incubated for 24 h before the cell viability was measured and compared with a control of non-treated cell.
Results: PMMA cements and organogels were able to generate heat in the range of 41–42 °C and displayed sustained release over 10 days. The release profiles were not influenced by the heat generated during a 25-min hyperthermia session at 6 mT, allowing further *in vitro* studies on the synergetic effects of hyperthermia and chemotherapy [2]. The heating power of the implants, so-called specific power loss, indicates the potential for hyperthermia-induced antitumoral effect [1]. Cement for intraosseous injection might provide some mechanical support to the weakened bone, in contrast to organogels which would be more suitable for soft tissue tumors. *In vitro* toxicity on PC3 cells shows preserved drug bioactivity, resulting in up to 40% and 75% cell death after 24 h exposure to the elution medium for cement and organogels, respectively.
Conclusions: Two different approaches for *in situ* forming implants were evaluated. Two types of implants were successfully loaded with doxorubicin and superparamagnetic nanoparticles, providing a sustained anticancer agent delivery and potential cytotoxic temperature. These data show within clinically acceptable parameters the feasibility of combining SPIONs for hyperthermia with local anticancer agent release.

Keywords: Hyperthermia, SPION, *in situ* forming implants, bone metastases, PC3.

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P-43

Overexpression of Adenosine Kinase Causes a Trypanostatic Effect in *Trypanosoma brucei*

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Introduction: *Trypanosoma brucei (T. brucei)* is the causative agent of Human African Trypanosomiasis (HAT; sleeping sickness). The existing drugs against HAT have severe side-effects, are expensive and need to be administrated over long periods by injection. Therefore safe and effective new medicines are required to treat this disease. Our previous research activities found that 4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl]-morpholine exhibits antitrypanosomal activity with an IC_{50} of 1 µM, and chemical proteomics identified *T. brucei* adenosine kinase (TbAK) as the intracellular target. Subsequent biochemical analyses found this compound to be a strong activator of TbAK, an important enzyme involved in the purine salvage pathway. The purine metabolism of *T. brucei* is a valuable drug target, since in contrast to mammals the parasites lack de novo purine biosynthesis.
Aims: The aim of the present project is the genetic and chemical validation of TbAK as the intracellular target and the applicability of TbAK hyperactivation as novel strategy to develop trypanocides.
Methods: We developed a tetracycline inducible *ak* overexpression strain of bloodstream-form (BSF) *T. brucei* since we hypothesized that overexpression of TbAK may compare to hyper-activation in presence of the activator. We also constructed a second tetracycline inducible overexpression strain harbouring an inactive TbAK (D299V) in order to exclude a possible toxic effect due to the presence of non-physiological high levels of TbAK. The phenotype of these cells was studied as well as their sensitivity towards the activator. In addition, we completed the study with an HPLC analysis of the intracellular purine levels.
Results: Growth of BSF *T. brucei* was reduced up to 75% within the first 24 h of TbAK overexpression compared to the wild type. The growth of these cells reached the same growth rate as the one of wild type cells after 3 days of induction. Addition of 1.5 µM

activator to the wild type cells showed a similar growth inhibition pattern as overexpression of TbAK except that growth remained reduced compared to wild type cells even after 3 days of induction. No synergistic effect was observed upon addition of 1.5 µM activator to the overexpression strain. Overexpression of the inactive TbAK showed a similar growth inhibition as wild type cells with activator. While the growth inhibition of the TbAK overexpression strain could not be measured, the IC_{50} of *T. brucei* overexpressing inactive TbAK was 1.4 ± 0.3 µM.
Conclusions: Overexpression of adenosine kinase causes a trypanostatic effect in *T. brucei.* Adenosine kinase may have an additional function besides converting adenosine to AMP since overexpression of inactive TbAK leads to growth inhibition as well.

Keywords: *Trypanosoma brucei,* adenosine kinase, purine.

P-44

Simultaneous Analysis of Cholesterol, LDL, Triglycerides and Lipid-Lowering Drugs from Dried Blood Spots

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Introduction: Dried blood spots (DBS) sampling represents an attractive alternative to classic venous sampling in bioanalysis. By collecting micro volumes of blood after a small finger prick, the DBS process affords numerous advantages that range from better shipment/storage logistics to a less invasive and more ethical sampling procedure. Initially used for the neonatal screening of metabolic disorders, DBS recently gave rise to a lot of interest in pharmaceutical and medical communities to support pharmacokinetic studies, clinical investigations or therapeutic drug monitoring.
Aims: Over the past decade, many publications have demonstrated the potential of DBS samples in the quantitative and qualitative analysis of various drugs based on liquid chromatography-mass spectrometry (LC-MS). In contrast, the use of this sampling support for the clinical chemistry is still unconventional [1]. The aim of this work was to develop an analytical strategy to simultaneously monitor from DBS samples cholesterol (CHL), LDL, triglycerides (TGL), and circulating concentrations of the most prescribed lipid-lowering drugs. These include simvastatine, pravastatine, atorvastatine, fluvastatine, rosuvastatine, and ezetimibe.
Methods: CHL, LDL and TGL were analyzed from a 10-µL dried plasma spot (DPS) using a conventional clinical chemistry system. Before analysis, DPS were punched out and were set into plastic tubes. The extraction was carried out with 120 µL of deionized water for 15 min under sonication at 30°C. The 6 lipid-lowering drugs were analyzed using the on-line DBS concept coupled with a conventional reversed phase LC separation with subsequent tandem mass spectrometric (MS/MS) detection [2]. Briefly, DBS samples (i.e. 5 µL) were set into a homemade prototype to be automatically extracted into the analytical system using the organic mobile phase. After their separation in a gradient elution mode, the analytes were detected in negative MRM mode with APCI source.
Results: Fifteen DPS samples from atherosclerotic patients were investigated for CHL, LDL, and TGL. Concentrations were then compared with values obtained in plasma collected by classic venipuncture. The results showed a good correlation between DPS and plasma concentrations for the three tested parameters. The on-line DBS LC-MS/MS method allowed for the rapid quantification of the 6 lipid-lowering compounds in the concentration range 5 to 500 ng/mL. The method was successfully applied to pharmacokinetics investigation performed on human patients.
Conclusions: Using approximately 25 µL of blood material, the developed strategy allowed for drugs and associated clinical parameters to be monitored simultaneously. In order to improve the patient comfort, this strategy seems to be particularly suitable for clinical investigations and treatment compliance.

Keywords: Dried blood spots, lipid-lowering drugs, cholesterol, triglycerides.

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P-45

Identification and Validation of Drug Targets in *Plasmodium Falciparum* – An Integrated Approach

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Introduction: Malaria is a tropical disease killing an increasing number of people worldwide. The parasite causing the deadliest form of malaria is *Plasmodium falciparum.* For several years now, this disease is developing resistance to current drugs, thus there is an urgent need for new treatments and new targets. Triclosan is an antibacterial and antifungal agent that was shown to inhibit the growth of blood stage of *P. falciparum in vitro* with an IC_{50} of 3 µM. However, its target in still unknown and needs to be elucidated.
Aims: It is the aim of this project to identify the putative target(s) of triclosan in *P. falciparum* by using chemical proteomics and two complementary in silico approaches, i.e. target-based inverse screening and ligand-based pharmacophore search.
Methods: For chemical proteomics, triclosan derivatives were attached to agarose beads at different positions. *P. falciparum* lysate was incubated with the affinity matrix and retained proteins were analyzed by LC-MS/MS. In silico inverse screening using the protein-based approach was carried out with the program PINTS while, with respect to the ligand-based approach, the program GOLD was applied to identify proteins potentially binding triclosan and its derivatives. For the subsequent modeling and docking for validation purposes the software packages GOLD and FlexX were used.
Results: While chemical proteomics did not succeed in defining potential targets, in silico inverse screening proposed several potential targets. Two amongst them, the heavy metal transporter 2 (PfMDR2) and the calcium-dependent protein kinase 2 (PfCDPK2), were confirmed by *in vitro* tests. Triclosan showed an IC_{50} of 17.4 µM on the ATPase activity of the ATP-binding cassette (ABC) of PfMDR2, and an IC_{50} of 34 µM on PfCDPK2.
Conclusions: This study shows that in silico approaches are able to identify potential targets of active molecules and represent a valuable complementary tool to chemical proteomics. In addition, subsequent *in vitro* evaluation of hits by suitable biochemical assays is essential to confirm prediction results.

Keywords: Malaria, triclosan, inverse screening, PfMDR2, PfCDPK2.

P-46

Patterns of Counselling Performed in Swiss Community Pharmacies while Dispensing Medication

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Introduction: In the prescribing-dispensing process, community pharmacists are the last point of contact in the healthcare chain and uniquely poised to deliver targeted counselling, to screen for compliance problems, and to improve medication compliance. We defined compliance as the extent to which the patient's behaviour matches *agreed* recommendations from the prescriber [1], and counselling as an approach that focuses on enhancing individual problem-solving skills for the purpose of improving or maintaining quality of health and quality of life [2].
Aims: To detect counselling patterns in community pharmacies with special focus on compliance counselling.
Methods: We developed a tally sheet based on published recommendations [3] and analysed systematically patient's counselling in 20 randomly selected community pharmacies in the region of Basel, Switzerland. Tacit observation was performed over one day and services provided to every client were manually recorded.
Results: Services spent by 39 pharmacists and 93 members of their staffs to 1866 clients were recorded during a total of 148 h in February and March 2010. Of the 3922 items dispensed, 44.6% were on prescription. Counselling was provided 1911 times to 795 clients, predominantly by staff members (69%), and statistically more often to patients with a prescription (Chi2=98.3; p<0.0001). Counsels were dispensed on the correct use of the drug (44%), its dosage (35%), its pharmaco-logical effects (15%), and specific compliance issues (6%). Patients with prescription obtained statistically more advices on compliance issues than patients with self-medication (Chi2 =14.6; p<0.001). Pharmacists engaged statistically more often in compliance counselling than the other members of their staffs (Chi2 = 27.3; p<0.001).
Conclusions: Approximately half of the clients (56%) received at least one explicit counselling during the dispensing process, predominantly those with prescription drugs. The content of 94% of the advices derived from the summaries of product characteristics, while only 6% treated specific compliance issues, which require specific communication skills. Pharmacy education and continuing education programs need to reinforce the importance of counselling on compliance issues to every patient, and to provide actual and future pharmacists as well as the pharmacy staffs with adequate communication skills and tools.

Keywords: Compliance, adherence, counselling, community pharmacy, observation study.

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P-47

A Novel Cyclosporin A Micellar Ophthalmic Formulation as an Alternative to Systemic Administration for Corneal Graft Rejection Treatment

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Introduction: Every year 120,000 corneal grafts are performed worldwide. For "high risk" patients the rejection rate is around 65% [1]. Immunosuppression by cyclosporin A (CsA) is usually used to prevent the rejection, with consequently systemic side effects.
Aims: The aim of this study was the development of a new CsA topical formulation based on polyethylene glycol-hexylsubstituted poly(lactides) (MPEG-hexPLA) copolymers and the investigation of the efficacy of this formulation for prevention of corneal graft rejection.
Methods: The CsA micelle formulation was prepared and characterized as reported in previous studies [2, 3]. The corneal penetration study was evaluated *in vivo* with CsA/DiO (3,3-dioctadecyloxacarbocyanine perchlorate, fluorescent marker) micelles prepared with Nile Red-MPEG-hexPLA fluorescent copolymers. After 7 instillations, the corneas were fresh flat-mounted and analyzed immediately by Confocal Laser Microscopy. The efficacy of CsA/MPEG-hexPLA micelle formulation was tested *in vivo* on a rat model of corneal graft rejection. Two groups were studied: 11 rats received 25 µL of 0.5% CsA micelle formulation 5 times a day during 14 days, and a control group consisting of 8 rats received a physiological saline solution. During the clinical observations (5, 7, and 13 days after surgery) three parameters were scored: corneal transparency, edema, and neovascularization. At the end of the study, ocular tissues and blood were recovered. CsA was quantified by UPLC-MS/MS.
Results: The novel ophthalmic formulation was a buffered isotonic solution, wherein the CsA concentration was 5.0 ± 0.1 mg/mL (corresponding to 25% of w/w loading) with a copolymer concentration of 30 mg/mL. After topical instillation of a fluorescent micelle formulation, the confocal fluorescent analysis showed that MPEG-hexPLA micelles were able to penetrate into the cornea, accumulating in the epithelium and in the stroma up to the endothelium, proving that these micelles were able to reach all corneal structures. The micelle capacity to accumulate into the cornea explains the good success rate obtained in the cornea transplantation study for the treated group, which was 73% whereas in the control group only 25% grafts were accepted. It has to be pointed out that the CsA concentration (around 8 mg) used in the present study was 4 times lower than in a systemic treatment used in a previous study [4].
Conclusions: MPEG-hexPLA micelles are able to deliver CsA into ocular tissues, with high corneal concentration after topical administration. They are effective in the prevention of corneal graft rejection in the animal model.

Keywords: Polymeric micelles, corneal transplantation, drug delivery system, cyclosporin A.

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P-48

Prevalence of Unreached Cardiometabolic Targets Among Treated Patients – Subanalysis of Data from a Community Pharmacy Screening Campaign in Switzerland

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Introduction: Screening for cardiometabolic risk factors provided by community pharmacies attract also patients already treated for cardiovascular risks. A previous retrospective analysis of data from 4380 subjects demonstrated that one third of patients with prescribed drugs for antihypertensive (AHT) and lipid modifying therapy (LMT) did not reach their biomarker targets as defined by guidelines [1].
Aims: This prospective study aimed at confirming the results of the pilot study. Data was collected within a refined screening campaign with standardised measuring methods and specific training of pharmacists.
Methods: In a screening campaign for cardiometabolic risk factors, blood chemistry, blood pressure (BP), waist circumference (WC), drug therapy and physical activity were assessed in Swiss pharmacies arranged in the group 'TopPharm' in April 2010. "Not on target" was defined for patients with a prescription for AHT having a systolic/diastolic BP ≥140 and/or ≥90 mm Hg (in diabetic patients ≥135 and/or ≥85 mm Hg), LDL-C >3.4 mmol/L for patients with LMT, and fasting glucose ≥7.2 mmol/L for patients with antidiabetic treatment (ADT). If WC was >88 cm for women or >102 cm for men the patient was defined as optimisable and involved appropriate counselling.
Results: From a total of 1347 screened subjects, 329 (24.4%) were eligible because they had a prescription for either AHT, LMT, ADT or any combination. Of 261 patients with AHT, 161 (61.7%) were not on target because they violated their BP criterion. LMT was prescribed in 122 patients, of which 38 (31.2%) were not on target. Glucose targets were not reached by 14 (48.3%) of 29 patients with ADT. Increased WC was evident in 183 (55.6%). Out of 68 patients with combined drug therapy 14 (20.6%) failed in reaching two of their target outcomes.
Conclusions: Screening campaigns unintentionally attract an important proportion of patients who fail to achieve treatment targets despite prescribed therapy. Thus, in addition to interventions for patients newly identified to be at risk for cardiovascular disease, validated interventions are needed to support community pharmacies in addressing contributing factors to therapy failure, such as non-adherence, unfavourable lifestyle, drug interactions, and improper dosing.

Keywords: Biomarker, cardiovascular risk factors, pharmaceutical care issues, therapy failure.

Reference:
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P-49

In Vivo Discovery of a Peptide that Reverses RNA Toxicity in Myotonic Dystrophy Models

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Introduction: An increasing number of dominantly inherited diseases are linked to expansion of tri-nucleotide repeats within specific genes, which ultimately produce toxic RNA molecules. Myotonic dystrophy type 1 (DM1) is a neuromuscular disease caused by the expansion of non-coding CTG repeats in the *DMPK* gene. In the mutant transcripts, CUG expansions form RNA hairpins that sequester splicing and transcription factors into ribonuclear inclusions in muscle, heart, and brain. These factors include the MBNL1 protein, which plays a key role in the development of DM1 symptoms.
Aims: There is currently no cure for DM1. We developed a transgenic *Drosophila* model that expresses expanded CUG repeats in a non-translatable RNA, with the objective of conducting chemical screens under *in vivo* conditions that identified molecules targeting toxic CUG transcripts.
Methods: Using this model, we screened a positional scanning combinatorial hexapeptide library (PS-CPL). Hexapeptides in the PS-CPL are small (<900 Da), formed by D-amino acids, which are not recognized by proteases. They were administered orally.
Results: We identified a hexapeptide, ABP1, which reduced CUG RNA nuclear inclusions, caused a MBNL1 subcellular redistribution, and suppressed CUG-induced phenotypes in different *Drosophila* tissues, including brain and muscle. ABP1 also reversed muscle histopathology and splicing misregulation of MBNL1 targets *Clcn1, Serca* and *Tnnt3* in DM1 model mice. The molecular mechanism underlying ABP1 activity relied on the induction of a conformational shift in the toxic CUG secondary structure, from double-stranded to predominantly single-stranded.
Conclusions: The screening of a PS-CPL under *in vivo* conditions allowed us to discover a peptide with anti-DM1 activity in fly and mouse models by targeting the core of CUG toxicity. This peptide represents a promising approach in the generation of new treatments for DM1 and other pathologies caused by toxic CNG repeat expansions.

Keywords: Drug discovery, non-coding RNA, RNA secondary structure, therapeutic peptides.

P-50

Micelle-Water Partition Coefficients: A Potential Alternative to Octanol-Water Partition Coefficients for Pharmaceutical Compounds

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Introduction: For its dissimilarity to biological membranes, traditional octanol-water partition coefficients have been questioned by researchers in recent years. The partition coefficient is a key parameter for *in vitro* acquisition of biopartitioning data for new drug candidates [1]. Lipophilicity determines the drugs fate in the body. Incorrect data can lead to poor prediction of absorption and distribution of drugs inside the body consequently achieving

low therapeutic or toxic levels. Micellar liquid chromatography (MLC) has recently gained advancement for the generation of more accurate partition coefficient data for pharmaceutical compounds [2].
Aims: (i) To develop a micelle-water partitioning model for compounds of pharmaceutical interest using MLC and (ii) to determine the effect of temperature and pH on the micelle-water partition coefficient.
Methods: Capacity factors (K') for each surfactant concentration (sodium dodecyl sulfate; 10–30 mM) using MLC were calculated to determine the micelle-water partition coefficient for commonly used NSAIDs (ibuprofen, ketoprofen and flurbiprofen) [2].
Results: For all three drugs, an increase in temperature has almost no effect on partitioning of these drugs and $logP_{MW}$ remained fairly constant over the range of temperature (data not shown here). In contrast, an increase in pH resulted in a decrease in $logP_{MW}$ for all 3 drugs (Table 1).

Table 1: $LogP_{MW}$ over the range of pH

Drugs	pH 3.0	pH 4.0	pH 5.0	pH 5.8	pH 6.4	pH 7.0
Ibuprofen	2.14 ± 0.03	2.04 ± 0.1	1.98 ± 0.01	1.47 ± 0.01	1.22 ± 0.02	0.21 ± 0.1
Ketoprofen	2.13 ± 0.01	2.05 ± 0.02	1.82 ± 0.01	1.23 ± 0.01	0.99 ± 0.03	0.42 ± 0.01
Flurbiprofen	2.20 ± 0.02	2.03 ± 0.01	1.92 ± 0.01	1.45 ± 0.02	1.11 ± 0.01	0.39 ± 0.01

This could be explained on the basis of dissociation of drugs. At pH below pKa, drug will be in its non-ionised form, therefore, it will favours lipophilic environment of the micellar core. Increasing the pH from acidic to basic will result in an increased ionisation of drugs, hence, allowing the drug molecules to reside in the aqueous environment, consequently a decreased $logP_{MW}$ for all 3 drugs was observed.
Conclusions: Owing to structural resemblance of micelles to biomembranes, MLC has been proven to be a potential alternative to the traditional octanol water system for the accurate measurement of a partition coefficient. In particular, data acquisition is quick and the results are fairly reproducible.

Keywords: Micelle-water partition coefficient, micellar liquid chromatography, NSAID.

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P-51

Pharmaceutical Analysis: Drug-Micelle Interactions

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Introduction: Isothermal titration calorimetry (ITC) is a powerful technique for acquiring thermodynamic information based on measurement of the heat that is generated or absorbed in an interaction between two molecules. Surfactants tend to form aggregates or micelle at a certain concentration known as critical micelle concentration (CMC). Micelles are of particular interest in this work as they resemble biological membranes [1].
Aims: Saturation of micelles using model drugs, CMC determination in the presence of model drugs.
Methods: Calorimetric titrations were conducted by stepwise injections of an aqueous drug solution into two micellar solutions, namely SDS and CTAB at 298K and 305K. Secondly, CMC of SDS was determined in the presence of three model drugs over the range of temperature (298K–322K).
Results: On the basis of aggregation number of surfactant, drug-surfactant ratio for each micelle was calculated. The results showed that as higher the hydrophobicity of drug, as more it will be entrapped within the hydrophobic core of the micelle (Table 1).

Table 1: Calculated amount of drug per micelle

Micelle	Temp. (K)	Number of drug molecules per micelle	
		Paracetamol (90 mM)	Diphenhydramine (120 mM)
SDS (20mM) (N ≈ 62)	298	58 ± 4 (Drug-surfactant ratio = 1:1)	73 ± 2 (Drug-surfactant ratio = 1.2:1)
CTAB (12mM) (N ≈ 61)	305	114 ± 4 (Drug-surfactant ratio = 2.2:1)	135 ± 4 (Drug-surfactant ratio = 2.2:1)

ITC was investigated as a potential new method for the accurate measurement of a change in the CMC of surfactant in the presence of drugs over the range of temperature. CMC values are given in Table 2.

Table 2: CMC measurement using ITC at various temperatures

Temperature (K)	Critical micelle concentration (mM) in the presence of drugs over the range of temperatures			
	Without drug	With paracetamol	With caffeine	With theophylline
298	8.5 ± 0.21	6.0 ± 0.15	5.7 ± 0.14	6.4 ± 0.16
304	6.7 ± 0.16	5.3 ± 0.13	5.3 ± 0.13	6.0 ± 0.15
310	7.1 ± 0.17	5.7 ± 0.14	7.1 ± 0.18	7.5 ± 0.18
316	7.5 ± 0.18	6.4 ± 0.16	7.8 ± 0.19	7.8 ± 0.19
322	8.2 ± 0.20	8.5 ± 0.21	8.5 ± 0.21	8.9 ± 0.22

Conclusions: ITC offers a suitable way to assess the partitioning of drugs into micelles. ITC has the potential to investigate complex drug-surfactant interactions.

Keywords: CMC, drugs, isothermal titration calorimetry, micelles, saturation limit.

Reference:
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P-52

Binding Characterization of the S128R E-Selectin Mutant

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Introduction: E-selectin is a cell adhesion molecule belonging to the C-type lectin family. It is transiently expressed on vascular endothelial cells upon stimulation by chemokines and plays an important role in leukocyte tethering during the inflammation process. In various patient studies, the S128R E-selectin polymorphism has been linked with an increased risk of developing various severe, mostly cardiovascular and autoimmune diseases. The mechanism by which this mutation alters the physiological role of E-selectin is not fully understood, as the mutation is located in the EGF-like

domain and does not interact with the carbohydrate recognition domain (CRD). Previous studies suggest that the E-selectin-S128R does no longer bind exclusively to the natural ligands sialyl Lewis^a and sialyl Lewis^x, but additionally to non-fucosylated trisaccharides (3'-sialyl lactosamine), heparin, and various protein ligands [1]. However, these studies did not directly show the interaction between the E-selectin-S128R and the non-natural ligands, but demonstrated binding indirectly by competitive inhibtion of the binding of E-selectin-S128R to K562 or HL-60 cells.
Aims: To determine the role of the fucose moiety for carbohydrates binding to E-selectin-S128R mutant with a target-based approach and to reveal possible implications for the design of E-selectin antagonists.
Methods: E-selectin-WT and E-selectin-S128R were cloned, expressed in CHO cells, and functionally purified. The presence of the mutation was confirmed by DNA sequencing as well as by LC-MS/MS. A target-based binding assay was used for the affinity measurement of sialyl Lewis$^{x/a}$, 3'-sialyl lactose, 3'-sialyl-*N*-acetyl lactosamine, heparin, and bovine fetuin. The glycan specificity profile was determined with a glycan array screening.
Results: With the target-based binding assay, the binding phenotypes of E-selectin-WT and E-selectin-S128R could not be distinguished, i.e., no mutant specific binding to 3'sialyl lactose, 3'sialyl lactosamine, heparin, and bovine fetuin was observed. The glycan array screening confirmed the necessity for the fucose moiety for both wild type and S128R mutant E-selectin.
Conclusions: Our results clearly suggest that E-selectin-S128R does not have an altered binding phenotype concerning the CRD. Consequently, the related diseases originate from a different mechanism without involvement of the CRD. Therefore, potential glycomimetic drugs acting as antagonists of E-selectin-WT are likely to be also suitable for E-selectin-S128R.

Keywords: E-selectin, polymorphism, S128R.

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P-53

Haptocorrin – The Key to Vitamin B12 Dependent Tumour Targeting?

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Introduction: In the human blood stream vitamin B12 (cobalamin, Cbl) is transported with two different transport proteins, namely haptocorrin (HC) and transcobalamin (TC). TC is known to transport cobalamin to peripheral tissue and is essential for its cellular uptake. In contrast, the physiological function of HC, a glycoprotein which carries the major part of the vitamin in circulation, is largely unknown. Tumours often express HC in large amounts and recently, we could show that a Tc-99m-labelled cobalamin derivative selectively binds to HC homes for tumours [1]. HC-selective radiolabelled molecules could therefore be interesting tools for tumour treatment or imaging.
Aims: Specific goals of this study are to explore the potential of radiolabelled HC-binding compounds as tools for tumour targeting.
Methods: We established two mouse models of human cancer with HC expression. Mice were injected with a Tc-99m-labelled, HC-selective Cbl-derivative and imaged with SPECT/CT. Accu-mulation of the tracer in tumors and organs was quantified by *ex vivo* biodistribution studies. We established recombinant expression of HC (rhHC) in HEK293 cells and purification of the protein. *In vitro* binding studies were performed with Tc-99m labelled cobalamin derivatives. Binding of the HC-selective drug Tc-99m-Cbl(PAMA4) was monitored by spectral analysis and gel shift assays. Crystals of the carbohydrate-trimmed protein were obtained by the sitting drop vapour diffusion technique. Diffraction data were collected from single crystals at beamline X06SA at the Swiss Light Source and the structure was solved with molecular replacement and refined to a resolution of 2.6Å.
Results: Biodistribution studies demonstrated specific accumulation of Tc-99m-Cbl(PAMA4) in mouse models of human melanoma (CLS-1) and lung adenocarcinoma (HCC827). Interestingly, CLS-1 tumors shed high amounts of HC into the blood stream, while HCC827 showed tumour-restricted expression of HC, which resulted in more favorable biodistribution of the tracer. Compared to reported TC-binding Cbl-derivatives, uptake in the kidneys was low. The protein rhHC was expressed and purified to >99% purity. The protein was characterized and has proven to bind Tc-99m-B12(PAMA4) with high affinity. Crystallisation of rhHC lead to structure determination of rhHC in complex with Cbl. Based on the structure, we were able to model binding of Cbl-derived drugs, for example Tc-99m-Cbl(PAMA4).
Conclusions: We demonstrated that HC-selective ligands specifically accumulate in HC-expressing tumours. In addition, we identified structural features of HC that account for ligand selectivity and can be exploited for future drug design.

Keywords: Haptocorrin, vitamin B12, tumour imaging.

Reference:
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P-54

Reflux Disease, Gastrointestinal Ulcer or Weight Loss in Patients with COPD

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Introduction: Peptic ulcer disease, gastro-oesophageal reflux disease (GORD) and weight loss have been associated with chronic obstructive pulmonary disease (COPD). Many studies, especially on peptic ulcer and weight loss, are cross-sectional or were done back in the 1960s or 1970s.
Aims: Our purpose was to learn more about GORD, ulcer, and weight loss in relation to COPD during longterm follow-up in recent years.
Methods: We conducted a case-control and a follow-up study using the UK-based General Practice Research Database to assess and compare the prevalence and incidence of GORD, peptic ulcer and weight loss in patients with COPD and in COPD-free patients during the period 1995–2005.
Results: We identified 35 772 patients with COPD and the same number of COPD-free patients. Incidence rates of GORD, peptic ulcer and weight loss in COPD patients were 59.2, 14.8 and 134.0 per 10 000 person years, respectively. The risk of weight loss was increased in patients with COPD compared to COPD-free patients (1.81, 95% CI 1.61–2.02), while the risk of GORD (OR 1.19, 95% CI 1.00–1.40) or peptic ulcer (OR 1.24, 95% CI 0.92–1.66) was similar in both groups.
Conclusions: The results provide further evidence that COPD is associated with weight loss, while there is no materially increased risk for ulcer or GORD associated with COPD.

Keywords: COPD, GORD, weight loss, peptic ulcer.

P-55

pKa Determination of Polyprotic Acids and Bases by ^{1}H-NMR

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Introduction: In drug discovery and development, pK_a values are of upmost importance for the prediction of pharmacokinetic and pharmacodynamic properties. The pK_a value is a key physicochemical parameter of compounds and has a direct impact on aqueous solubility as well as permeability through biological membranes. Ionized molecules show an increased polarity compared to neutral molecules and therefore tend to be more soluble but less permeable. To date, different methods for the determination of pK_a values are available, including potentiometric, UV-spectroscopic, and capillary electrophoresis techniques. An additional option is provided by nuclear magnetic resonance (NMR) spectroscopy.
Aims: pK_a determinations by ^{1}H-NMR spectroscopy have been reported for numerous, but mostly isolated cases. The aim of this study was to develop an easy applicable standard operating procedure (SOP) and to determine scope of limitations of pK_a determination by ^{1}H-NMR. For this purpose, the pK_a values were experimentally determined for a broad set of structurally diverse compounds and the results compared to *in silico* predictions and reference values from literature.
Methods: The underlying principle of the pK_a determination by ^{1}H-NMR is the alteration of chemical shifts of magnetic nuclei depending on the protonation state of an adjacent acidic or basic site. When these chemical shifts are plotted against the pH, the inflection point of the resulting sigmoidal curve defines the pK_a.
Results: Test compounds with two or three ionizable protons were determined. A comparison of the results with reference and computed values showed excellent correlation.
Conclusions: The utility of the ^{1}H-NMR pK_a determination could be demonstrated on compounds with up to three ionizable centers applying a general SOP.

Keywords: pK_a determination, ^{1}H-NMR spectroscopy, physicochemical properties.

P-56

Vaginal Immunization Using Virosomal *Candida albicans* rSap2 vaccine: Toxicity and Immunogenicity Studies in Animals

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Introduction: To prevent the increasing number of recurrent vulvovaginitis in women, a strong vaginal immunity is required as the first line of defence, meaning both systemic and local immunization, as the vaginal mucosa is the main portal of *Candida* infection. Virosomes, defined as reconstituted influenza virus envelopes comprising the viral surface proteins hemagglutinin (HA) and neuraminidase (NA) but lacking the viral genetic material of the genuine pathogen, were able to elicit both humoral and cellular immune response in previous studies [1]. A new promising vaginal formulation for virosomes *C. albicans* rSap2 (secretory aspartyl proteinase 2) antigen, which is critically required for *Candida* infections, was developed to deliver rSap2 antigen to the vaginal mucosa.
Aims: Based on preliminary *in vitro* analysis, studies on the *in vivo* release, toxicology and immunological assays in New Zealand rabbits and Göttingen minipigs were performed under GLP conditions.
Methods: A suitable formulation for vaginal application [2, 3] of virosome-based vaccine containing rSap2 was developed. After intravaginal administration of capsules containing virosomes conjugated with rSap2 in both animal models, local (vaginal secretion), and systemic (serum) antibody responses (anti-rSap2 and anti-HA) were induced. The samples were analysed by ELISA.
Results: A suitable, patient-friendly virosome delivery system for vaginal application was developed. No adverse events related to the application of capsules were observed. In minipigs, no clinical signs were observed and hematology and blood chemistry parameters remained within the normal range. No vaccine-related macroscopic or histopathological changes were detectable in the vagina of rabbits. Despite suboptimal sampling time, local and serum antibody specific responses in minipigs as well as serum antibody response in rabbits were obtained.
Conclusions: The developed formulation for vaginal immunization with virosomes loaded with rSap2 did not show any local or systemic toxic effect. Furthermore, these vaccinations induced specific antibody responses in two animals species. Based on the previous encouraging results, a phase I clinical trial was initiated in 2010.

Keywords: Vaginal administration, candidiasis, virosomes, vaccine, rSap2.

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P-57

Mechanisms of GRK2-Mediated Control of Cell Proliferation

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Introduction: G-protein-coupled receptor kinase 2 (GRK2) exerts an indispensable role in the desensitization of G-protein-coupled-receptors (GPCRs). By phosphorylation of an activated GPCR, GRK2 induces the recruitment of β-arrestin and thereby uncouples the receptor from the G-protein. GRK2 also plays an essential role in cell proliferation and cell growth because mice lacking the gene for GRK2 are not viable, although underlying mechanisms are less clear.
Aims: To analyze the role of GRK2 in cell proliferation, we established a cellular model.
Methods: GRK2 or the kinase-deficient GRK2-K220R mutant were stably expressed in human cells and cell proliferation was determined. Concomitantly, cells were expanded *in vivo,* in immunodeficient NOD.Scid mice. Gene expression changes induced by GRK2 or GRK2-K220R were analyzed by whole genome microarray gene expression profiling. Immunohistology methods were applied to determine activated MAPK.
Results: To analyze the role of GRK2 in cell proliferation, we established a cellular model. GRK2 or the kinase-deficient GRK2-K220R mutant were stably expressed in human embryonic kidney (HEK) cells. However, GRK2 or GRK2-K220R did not change the cell proliferation rate of HEK cells. In contrast to *in vitro* cultivated cells, GRK2-dependent cell proliferation control was detected when cells were expanded *in vivo,* in immunodeficient NOD.Scid mice. While GRK2 expression led to a slightly reduced tumor mass, GRK2-K220R expression increased the tumor mass relative to control cells. Microarray gene expression profiling showed that GRK2-K220R had strongly increased the expression of MAPK target genes such as FOS. In

agreement with MAPK activation, nuclear accumulation of activated phopho-ERK1/2 was detected by immunohistology. Similarly to *in vivo*-expanded HEK cells, GRK2-K220R also enhanced the proliferation of squamous cell carcinoma A431 cells upon *in vivo* expansion.
Conclusions: Taken together, *in vivo* cell expansion reveals GRK2-mediated cell proliferation control.

Keywords: G-protein-coupled receptor kinase 2, cell proliferation, MAPK pathway, squamous cell carcinoma, tumor mass.

P-58

Symptoms of Heart Failure Induced by Cellular ATP Depletion

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Introduction: Ischemic heart disease induced by atherosclerosis is a major risk factor promoting the development of heart failure. However, mechanisms underlying the transition to heart failure in patients are incompletely understood. Cardiac ATP depletion is a characteristic feature marking the transition to heart failure in patients.
Aims: We analyzed the potential involvement of cellular ATP depletion in the induction of heart failure symptoms.
Methods: To investigate whether ATP depletion could contribute to the induction of heart failure we induced cellular ATP depletion *in vivo* by mild inhibition of the respiratory chain with cystamine. Apolipoprotein E- (ApoE-) deficient mice were chosen for cystamine treatment, because ApoE-deficient mice are prone to atherosclerosis and thereby mimic the risk profile of patients.
Results: After 4 weeks, cystamine treatment had significantly reduced cardiac ATP levels of ApoE-deficient mice. In addition to cardiac ATP depletion, cardiac histology revealed that cystamine had induced the development of cardiac hypertrophy with dilation. Concomitantly, signs of heart failure were detected by echocardiography as evidenced by a strongly reduced ejection fraction (< 30%). Whole genome microarray gene expression profiling of failing heart tissue relative to control tissue showed that the onset of cystamine-induced heart failure was accompanied by a strong up-regulation of heart failure-promoting genes of the cardiac lipid metabolism.
Conclusions: Taken together, our experiments show that cellular ATP depletion may contribute to the development of heart failure symptoms *in vivo.*

Keywords: Heart failure, ATP depletion, echocardiography, apolipoprotein E, microarray gene expression profiling.

P-59

Bryophyllum pinnatum Press Juice – The Effect on the Contractility of Porcine Detrusor *In Vitro*

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Introduction: The incidence of urinary incontinence (UI) caused by overactive bladder (OAB) occurs at all ages but increases with rising age. The costs of UI are enormous because of low compliance to the standard anticholinergic therapies [1]. To minimize these costs, there is a need for successful treatment with a minimum of adverse effects. *Bryophyllum pinnatum* press juice (BPJ) is used for tocolysis and shows few adverse effects [2].
Aims: We wanted to investigate the relaxant and inhibitory effects of BPJ on the contractility of porcine detrusor strips *in vitro.*
Methods: Strips of porcine detrusor were taken from the bladder wall and tested in a myograph system chamber aired with O_2/CO_2 at 37 °C. Concentrations were induced by carbachol (50 µM) and thereafter relaxation measurements were performed. For the inhibitory effect measurements, electrical field stimulation (32 Hz, 40 V) was used to induce contractions. Recordings were obtained in the absence and presence of increasing concentrations of BPJ (0.1–10%) as well as experiments with oxybutynin (10^{-7}–10^{-3} M) as a reference substance were performed.
Results: BPJ as well as oxybutynin relaxed carbachol pre-contracted porcine detrusor strips significantly. The maximum effect was 19.7 ± 3.5% ($p < 0.05$) and 84.6 ± 3.2% ($p < 0.001$), respectively. In inhibition experiments BPJ as well as oxybutynin were able to reduce the electrically induced contractions of porcine detrusor significantly with a maximum effect of 69.7 ± 10.2% ($p < 0.001$) and 96.2 ± 1.4% ($p < 0.0001$), respectively.
Conclusions: Our investigations clearly showed that BPJ is able to relax carbachol-induced contractions as well as inhibit contractions induced by electrical field stimulation. However, the effect was lower compared with the reference substance oxybutynin. The currently used anticholinergic drugs are unpopular. It is therefore important to continue *in vitro* experiments as well as clinical studies with BPJ.

Keywords: Overactive bladder, *Bryophyllum pinnatum.*

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P-60

Non-Invasive Transdermal Iontophoretic Delivery of Functional Proteins – A New Therapeutic Approach

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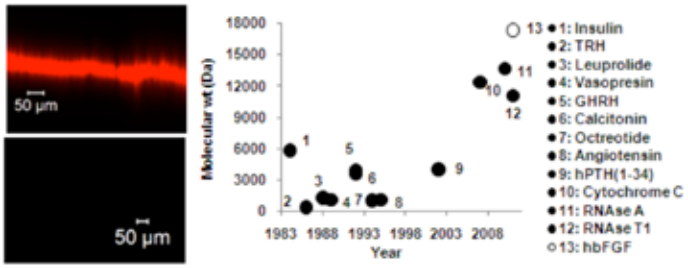
Introduction: The physicochemical properties and stability requirements of proteins mean that they are routinely delivered by injection. Transdermal iontophoresis offers a controlled non-invasive alternative, but until recently it was not considered possible to deliver proteins with this technique [1]. However, in addition to demonstrating feasibility, it is also essential to confirm the structural and functional integrity of proteins post-delivery.
Aims: To demonstrate the feasibility of using transdermal iontophoresis to deliver intact functional proteins – ribonuclease A (RNase A; [2]), ribonuclease T1 (RNase T1; [3]) and human basic fibroblast growth factor (hbFGF; [4]) – non-invasively into and across the skin.
Methods: Experiments were performed using vertical diffusion cells. The donor chamber contained 1 ml of each formulation – RNase A (50 µM; pH 6), RNAse T1 (50 µM; pH 7.8) and hbFGF (29 µM; pH 7.4). Constant current (0.5 mA/cm^2) was applied for 8 h and the receiver compartment sampled hourly. After terminating current application, bound protein was extracted from the skin. RNase A and RNase T1

delivery was determined by a methylene blue-based enzymatic assay, providing simultaneous quantification of transport and confirmation of activity. hbFGF was quantified by ELISA and functionality confirmed using HFF and NIH 3T3 cell lines. Skin distribution of labeled hbFGF was visualized by confocal laser scanning microscopy (CLSM).
Results: Cumulative permeation of RNAse A and RNAse T1 was 252 ± 19 and 142 ± 62 µg/cm^2, respectively. Structural integrity was confirmed by SDS-PAGE and MALDI-TOF spectra. Skin deposition of RNase A and RNase T1 was 206 ± 20 and 9 ± 3 µg/cm^2, respectively (~24 fold higher for cationic RNase A). The methylene blue assay confirmed that enzymatic activity was retained after skin transit. Cumulative permeation and skin deposition of hbFGF were 16 ± 7 and 78 ± 37 µg/cm^2, respectively. Increased proliferation of HFF and NIH 3T3 cells confirmed its activity. CLSM images showed hbFGF distribution in the skin (Fig. below left), desirable for dermatological indications. Delivery of hbFGF across porcine and human tissue was statistically equivalent (16 ± 7 and 13 ± 1 µg/cm^2, respectively). To-date, hbFGF is the largest protein delivered non-invasively across intact skin (Fig. below right).



Conclusions: The results demonstrate that iontophoresis can successfully deliver intact, functional proteins non-invasively across the skin. The technology may provide a realistic alternative to parenteral administration of proteins for local and systemic therapy.

Keywords: Dermatology, protein delivery, non-invasive, transdermal, iontophoresis.

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P-61

Antiplasmodial Bisaboleneoxide Derivates from *Artemisia persica* Bioss and Determination of Absolute Configuration by Theoretical Calculation of Electronic Circular Dichroism (ECD) via Density Functional Theory

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Introduction: Iran has a long medical history and traditional knowledge of plant remedies. However, very few of these plants have been studied from a pharmacological or phytochemical point of view. As part of a phytochemical study of endemic plants of Iran, and an ongoing screening for new antiprotozoal natural products, we have screened 102 extracts obtained from plants used in Iranian antimalarial remedies. An ethylacetate extract of *Artemisia persica* (Asteraceae) showed notable antiplasmodial activity, with 78.6% inhibition at 0.8 µg/mL.
Aims: Isolation and structure elucidation of active compounds against *Plasmodium falciparum* K1 strain by using HPLC-based profiling.
Methods: The active compounds in the extract were tracked by HPLC-based profiling. Subsequent preparative isolation of the peaks in the bioactive windows resulted in 5 new bisabolene sesquiterpene diesters. Structure elucidation was achieved by 1D and 2D NMR experiments. Relative stereochemistry was established by NOESY and NOE difference measurement. The absolute configurations of the constituents were assigned by comparison of their simulated ECD spectra by using density function theory (DFT) in gas phase (B3LYP/6-31G**) and methanol (B3LYP-SCRF /6-31G**) and comparison with experimental CD spectra.
Results: The isolated sesquiterpenes exhibited moderate antiplasmodial activity with IC_{50}s ranging from 2.8 to 20 µM, and selectivity index (SI) in L-6 cells of 3.7 to 11.9.
Conclusions: The classical approach for identifying active natural products in complex mixtures is bioassay guided isolation, which is tedious and time-consuming and, hence, not suited for a medium- to high-throughput setting. HPLC-based activity profiling is an effective strategy to deal with such limitations in capacity and to speed up the discovery of new leads.

Keywords: *Artemisia persica, Plasmodium falciparum,* bisabolene sesquiterpene.

P-62

TRPM4 Variants in Patients with Childhood Atrio-Ventricular Block

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Introduction: Atrio-ventricular block (AVB) is a condition in which conduction of cardiac impulses from the atria to the ventricles is impaired. Prevalence of non-immune isolated congenital or childhood AVB is very low and its etiology remains unknown. Recently, mutations in the TRPM4 gene, encoding a calcium-activated non-selective cation channel, were described in patients with a familial form of progressive cardiac conduction disease (PCCD).
Aims: To find causal mutations of childhood AVB and to dissect their molecular and cellular mechanisms.
Methods: High Resolution Melt (HRM) and direct DNA sequencing; transient transfection in HEK293 cells; cell surface biotinylation; immunoblot; deglycosylation assay; whole-cell configuration patch clamp.
Results: In the present study, we evaluated the possible involvement of TRPM4 in congenital and childhood AVB. TRPM4 was screened by HRM and direct DNA sequencing in 91 children with AVB. We identified 10 genetic variants in 14 children (15.4%), 2 of which have already been described in PCCD. By performing immunoblot experiments using lysates from HEK293 cells transiently transfected with wild type and mutant constructs, the TRPM4 channel was

found to be expressed as a double band corresponding to fully and core glycosylated forms of the channel. Two of 6 variants chosen for further studies showed a significant decrease (>50%) in their protein expression levels. mRNA levels were unchanged. Incubation of HEK293 cells for 24 h at lower temperature (28 °C) rescued their expression, suggesting that these variants may cause channel misfolding, thus resulting in trafficking problems. Intriguingly, patch clamp using whole-cell configuration showed a 2-fold increase in TRPM4 current in the variants despite the decrease in their protein expression levels.
Conclusions: The results above suggest that TRPM4 is an important susceptibility gene for congenital AVB. The molecular and cellular mechanisms by which these variants alter the function of TRPM4 channel remain to be studied.

Keywords: TRPM4, childhood AVB, immunoblot, patch clamp.

P-63

Amino Acid Transporters of Trypanosoma brucei

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Introduction: In Trypanosomatids, exogenous amino acids are required for protein synthesis, osmoregulation, as energy source and as differentiation signals [1]. However, little is known on the transporters mediating import of amino acids and their role for parasite growth, development, and pathogenicity. Amino acid transporters may also represent potential drug targets or drug delivery systems. For example, *Trypanosoma brucei* AAT6, a member of the amino acid/auxin permease (AAAP) family was recently identified as entry gate for DFMO [2], a drug for stage 2 human African trypanosomiasis.
Aims: Identification of genes coding for potential amino acid transport proteins in the genome of *T. brucei.* Investigating their role for the parasite, e.g. in uptake of essential amino acid, uptake of amino acid as energy source or osmoregulators. Characterizing substrate selectivity, including toxic analogs, and evaluating a potential role as drug targets or drug targeting systems.
Methods: The genome of *T. brucei* was analyzed with the hmmer-3.0 program and profiles for protein families associated with amino acid transport. *Saccharomyces cerevisiae* mutants deficient in amino acid uptake were transformed and screened for growth on selective media. Role of the transporters in parasites is being evaluated by generation of knock-down trypanosomes using RNAi. Functional characterization of the transporters was determined in heterologous expression systems i.e. by uptake of radiolabeled amino acids in *S. cerevisiae* mutants or voltage clamping of *Xenopus laevis* oocytes.
Results: 48 genes coding for putative amino acid transporters were identified. Consistent with the results of [3], 47 were members of the amino acid/auxin permease (AAAP) family and one potential amino acid transporter was a member of the amino acid-polyamine-organocation (APC) transporter family. 39 ORFs were amplified by PCR and expressed in different *S. cerevisiae* mutants. 14 ORFs were able to restore growth on selective concentrations of one or several amino acids. B17 mediated arginine transport when expressed in *S cerevisiae* and was essential in procyclic trypanosomes. Expression in oocytes revealed that AAT6 is a low selective amino acid transporter. Its main substrate was proline with a $K_{0.5}$ of approximately 1.2 mM (pH 7.4, V_m –80 mV). D-proline evoked similar currents as L-proline and also DFMO induced currents were measured.
Conclusions: 48 genes in the genome of *T. brucei* were found to encode potential amino acid transporters. 14 of 39 ORFs tested were able to restore growth of *S. cerevisiae* mutants on one or more amino acids. AAT6 showed very low substrate selectivity, transporting a number of amino acids, D-proline and DFMO. B17 transported mainly arginine, and was essential in procyclic trypanosomes.

Keywords: Amino acid, transport, *Trypanosoma brucei*, proline, arginine.

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P-64

Identification of Stimulators of Lymphatic Function derived from Natural Products

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Introduction: The lymphatic system is involved in the maintenance of tissue fluid homeostasis, the uptake of dietary fats and the immune response, and it also plays a major role in pathologic conditions, such as cancer metastasis, lymphedema and inflammatory diseases. Recent findings suggest that the modulation (inhibition or stimulation) of the lymphatic system might be a new strategy for the therapy of those conditions.
Aims: We aim to identify stimulators of lymphatic function and lymphangiogenesis, i.e. the formation of new lymphatic vessels, and to characterize their mode of action in order to develop new modulators of lymphangiogenesis with potential clinical applications.
Methods: *In vitro* proliferation, migration and sprouting assays were used for the screening of a plant extract library and for the characterization of the hits. We applied different biochemical methods in order to identify the active ingredients of extracts and the signaling pathways involved in mediating the observed effects.
Results: We screened a library of more than 600 plant extracts and identified a plant extract that strongly induced lymphangiogenesis *in vitro.* This extract potently induces the proliferation, migration and sprouting of lymphatic endothelial cells (LEC). The stimulation of LECs was dependent on the concentration of the extract. In contrast, blood vascular endothelial cells were not significantly stimulated by the extract. To assign the activity of the extract to one or more active compounds, it was fractionated using HPLC. We were able to identify the active substance class and confirmed the stimulating effect of the isolated compounds on LECs using *in vitro* assays. Furthermore, we could show that the effect is mediated via the ERK1/2 pathway. Currently, we are developing a suitable formulation for the topical application of the most potent compound.
Conclusions: Taken together, we have identified a plant extract that potently induces proliferation, migration and sprouting of LECs *in vitro.* Furthermore, we were able to assign this effect to one class of substances that is specific for this plant and could identify the responsible molecular pathway. Future studies will evaluate the *in vivo* activity of the identified compounds in mouse models of human diseases related to the lymphatic system.

Keywords: Natural products, lymphangiogenesis, (lymphatic) endothelial cells.

P-65

Enhanced Permeability to Liposomal Formulations on a BBB-PAMPA Model

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Introduction: The central nervous system (CNS) is protected by the blood-brain barrier with its primary function to maintain constant physicochemical conditions in the brain. Tight junctions, low vesicular transport and high metabolic activity achieve this barrier function. It is well known that around 80% of commercial drugs cross membranes by passive transport, it represents in fact the main transport way that occurs in the body, depending on compounds physicochemical characteristics and molecular weight. In CNS the passive passage is hampered by efflux proteins such as the P-glycoprotein, owning a defense function against external environment and xenobiotics. These proteins are also responsible of multidrug resistance (MDR) leading to failure of therapies or decreasing of their therapeutic effectiveness. As in some case of CNS cancers and disorders treatments, low bioavailability is due to poor water solubility and to the difficulty to reach the target site in effective dose. Therefore, to reach the effective dose, it is necessary to administrate higher doses of drug, leading to adverse side effect. Thus, doses should be reduced to reduce adverse effect and an efficient drug delivery for a difficult target as CNS remains a challenge. Liposomal formulation can be helpful to protect some drugs against efflux proteins and, thus, overcome the MDR problems if their passive permeation will be sufficient. Moreover, when a liposomal encapsulated drug is administrated, prolonged concentration profiles are observed partly by lowering the metabolic clearance but also due to the tissue distribution since their permeability across the barriers is modulated.
Aims: Since liposomal formulations are successfully used to overcome MDR complications and represent a therapeutic approach to deliver efficiently compounds to the BBB target sites, an *in vitro* model able to rapidly screen passive permeability of liposomal formulations should be therefore of great interest in drug discovery stage.
Methods: Liposomal formulations were prepared in order to encapsulate five compounds owing different physicochemical characteristics in term of solubility and lipophilicity. After characterization, these preparations were tested on the BBB-PAMPA model [1] in order to evaluate its ability to predict passive BBB permeability of encapsulated compounds.
Results: Liposomal encapsulations of some drugs having a CNS activity have shown an increase in permeability on the BBB-PAMPA model, compared to the free drug, presumably by passive permeation of liposomes.
Conclusions: These preliminary results open new perspectives in the evaluation of the permeability of liposomal formulations with the advantages of *in vitro* BBB-PAMPA, a model characterized by its low-cost and less labor intensive compared to cellular models.

Keywords: BBB-PAMPA, liposomes, CNS diseases, bioavailability.

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P-66

Short-Term Memory Impairment in Mice Caused by Hippocampal Downregulation of the Neuronal Monocarboxylate Transporter MCT2

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Introduction: MCT2 is the predominant neuronal monocarboxylate transporter involved in the transport of lactate, an energy substrate which seems to play a key metabolic role in the CNS. Previously, we found that brain-derived neurotrophic factor (BDNF), an essential element in the mechanism of synaptic plasticity, enhances MCT2 expression *in vitro* in cultured cortical neurons and in synaptosomal preparations [1] as well as *in vivo* by acute injection in the CA1 hippocampus [2]. In addition, it was demonstrated that MCT2 and GluR2 (an AMPA receptor subunit) are colocalized in the post-synaptic density [3] and associated in a common trafficking route [4]. We therefore hypothesize that changes in MCT2 expression participate in the process of synaptic plasticity.
Aims: We highlight the importance of monocarboxylate transport into neurons in spatial memory formation and the coupling between spatial memory and energy metabolism.
Methods: In the present study, we assessed the effect of hippocampal MCT2 downregulation on the proficiency of learning and memory of spatial tasks. Bilateral stereotaxic injections of lentiviral vectors were performed into mouse hippocampal CA1 areas. Twelve mice were injected with a lentivirus expressing the siMCT2 (MCT2 knockdown mice) and 12 mice with a lentivirus expressing a non-sense siRNA. (control siRNA mice) . Spatial working and reference memory abilities were evaluated on a radial arm- and a Morris water maze, while the dynamics of episodic memory formation was assessed in a passive avoidance task.
Results: MCT2 knockdown resulted in clear short-term memory impairments, yet with a mild impact on long-term spatial reference memory.
Conclusions: These results indicate that MCT2 is involved in the mechanisms of working memory, and support the notion that short-term and long-term memory can be supported by independent molecular mechanisms.

Keywords: Lactate, synaptic plasticity, hippocampus, memory.

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P-67

Endocannabinoid Content in Fetal Bovine Sera – Unexpected Effects on Mononuclear Cells and Osteoclastogenesis

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Introduction: The major endocannabinoids (ECs) N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) and other N-ethanolamines act as full and partial agonists at CB1, CB2, GPR55, PPAR and TRPV1 receptors to various degrees. These receptors are also expressed in immune cells like monocytes/macrophages where they regulate different cellular processes.
Aims: In this study, potentially bioactive lipids in commercially available fetal bovine sera (FBS) were quantified by gas chromatography-mass spectrometry (GC/MS) to study their potential impact for cell culture systems and experiments involving the endocannabinoid system.
Methods: The samples were analyzed by GC/MS using an Agilent 6890N GC equipped with a 30 m HP-5MS column and a 5975C EI-MS with triple-axis detector. Specific ions were chosen for selected ion monitoring and deuterated standards used. After derivatization with pentafluorobenzylbromide/*N,N*-diisopropylethylamine and dimethylisopropylsilyl imidazole the fatty acids were quantified by GC/MS [1]. Lower limits of quantification (LLOQ) on column for the measured compound were as follows: AEA 40 pg, 2-AG 2 ng, arachidonic acid (AA) 4 ng, palmitoylethanolamide (PEA) 20 pg, oleoylethanolamide (OEA) 60 pg, stearylethanolamide (SEA) 60 pg, and prostaglandin E2 (PGE2) 50 pg. Endocannabinoid high-content sera were compared to endocannabinoid low-content sera in different cellular systems, such as GM-CFS/RANKL-stimulated osteoclastogenesis from primary human PBMCs. Osteoclastogenesis assays using primary human monocytes/macrophages were performed as previously described [2].
Results: We found that several commercial FBS contain bioactive amounts of 2-AG (100–250 ng/mL) and potentially also PEA, but negligible amounts of AEA and SEA. Residual 2-AG in FBS activated primary macrophages and increased migration and RANKL-stimulated osteoclastogenesis. Furthermore, 2-AG high-content sera containing medium specifically up-regulated LPS-stimulated IL-6 expression in U937 cells. Polymyxin B beads selectively removed 2-AG and partially also AEA and arachidonic acid from sera but not the other N-ethanolamines [3].
Conclusions: Low 2-AG concentrations in cell cultures may significantly modulate cellular processes and mononuclear cells expressing strong CB receptor surface expression may be particularly sensitive towards 2-AG high-content FBS. Therefore, ECs in FBS should be monitored and controlled in biological experiments.

Keywords: Endocannabinoids, cell culture, osteoclastogenesis, macrophages.

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P-68

Evidence Supporting the Existence of an Endocannabinoid Membrane Transporter Involved in Cellular AEA and 2-AG Uptake

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Introduction: Endocannabinoids (ECs) are key mediators involved in many physiological and pathological conditions in CNS and peripheral tissues where they exert biological activities by interacting with extracellular and intracellular targets. The effects of ECs are regulated by cellular biosynthesis, release, re-uptake, trafficking and enzymatic metabolism. Unlike the clear knowledge about the biosynthetic and metabolic pathways of ECs, their cellular re-uptake is still a debated issue with several mechanisms proposed.
Aims: One of the principal issues in elucidating the transport mechanism is the tight inter-play between ECs plasma membrane movement and their rapid and almost complete cellular cytoplasmic cleavage by the enzymes FAAH and MGL. We therefore designed a new methodology.
Methods: Radioactivity assays, TLC and GC/MS methods for determination of ECs in whole cells (U937 human monocytic cell line) were developed to investigate the intracellular and extracellular level of AEA, 2-AG and their degradation products. This new combined approach has been applied to investigate the uptake and hydrolysis of AEA and 2-AG when co-incubated or when incubated alone in presence of specific endocannabinoid membrane transporter (EMT) inhibitors, enzymatic inhibitors (FAAH, MGL) and combinations of them.
Results: (1) Radioactivity-based results show additive and super-additive effects in AEA uptake inhibition and AEA extracellular accumulation when combining an EMT inhibitor with a FAAH inhibitor in comparison to the effect of the FAAH inhibitor alone. (2) GC/MS quantification showed an increase of the intracellular AEA level following FAAH inhibitor treatment while EMT inhibitor and the combination led to a reduction. (3) TLC analysis of the intracellular radioactivity showed that the vast majority of the signal derives from [^{3}H]-ethanolamine incorporated into phospholipids and not from intact [^{3}H]-AEA. (4) EMT inhibitors were tested in cells expressing low- or high-level of fatty acid binding proteins (FABPs, recently identified as AEA intracellular carrier proteins) showing no difference in AEA uptake inhibition potency, while the highly FABPs-expressing cells were much more sensitive to a selective FABPs-inhibitor. (5) Co-incubation assays with AEA and 2-AG showed that these molecules compete for the same membrane transporter and that it is more selective towards AEA. (6) The MGL inhibitor JZL184 induced an intracellular accumulation of 2-AG, while EMT inhibitors reduced the intracellular level in both radioactivity assays and GC/MS quantification. (7) TLC analysis showed no incorporation of [^{3}H]-glycerol into phospholipids.
Conclusions: The results obtained by using radioactivity assays, GC/MS and TLC analyses in presence of different FAAH, MGL and EMT inhibitors alone or in combination provide evidence in favor of the presence of a common EMT involved in AEA and 2-AG cellular uptake. Our data indicate that AEA and 2-AG compete for the same membrane transporter (EMT) when co-incubated and that the transporter possesses a higher selectivity towards AEA.

Keywords: Endocannabinoids, membrane transport, methodology.

P-69

Analogues of Resorcylic Acid Lactone L-783277: Syntheses and SAR Studies

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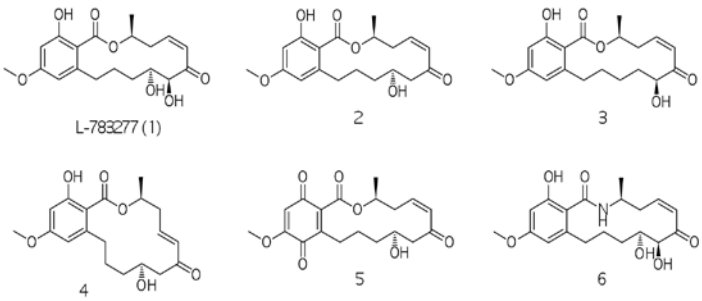
Introduction: Resorcylic acid lactones (RALs) are mycotoxins produced by a variety of different fungal strains, of which those containing a *cis*-enone moiety have been reported to be potent, irreversible kinase inhibitors [1]. L-783277 (**1**) was first isolated from *Phoma* sp. (ATCO 74403) by Zhao *et al.* at Merck Research Laboratories in 1999 and shown to inhibit MEK1 *in vitro* with an IC_{50} value of 4 nM [2]. The *cis*-enone moiety **1** is essential for its kinase-inhibitory activity, which is due to Michael addition of a cysteine residue present in the ATP-binding pocket. A corresponding cysteine residue is found only in a subset of kinases, comprising about 10% of the total human kinome. The first total synthesis of L-783277 (**1**) has recently been published by our group [3].

Aims: As part of our ongoing SAR studies around L-783277 (**1**), this contribution reports the syntheses and biological evaluation of the deoxy analogues **2**, **3** and **4** [4], the benzoquinone analogue **5** as well as the progress in the synthesis of lactam analogue **6**.

Results and Conclusions: Intriguingly, the 5'-deoxy analogue of L-783277 (**2**) retains almost the full kinase inhibitory potential of the parent natural product L-783277 (**1**). In contrast, 4'-deoxy analogue **3** and C7'-C8' *E* derivative **4** are significantly less active [4]. These data show that the 5'-hydroxy group is a functionally less relevant or even irrelevant structural feature of L-783277 with respect to kinase inhibition. This remarkable finding on the activity of **2** broadens the perspective for the design of new RAL based inhibitiors using monohydroxy scaffold **2**.



Keywords: Resorcylic acid lactone, L-783277, synthesis, SAR.

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Mikrobiologie • Biofilme

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Gespräch mit Prof. em. Dr. Bernhard Guggenheim, Institut für Orale Biologie, Zahnmedizinisches Zentrum der Universität Zürich, Zürich

Das Gespräch zeigt auf, dass für das Fach Mikrobiologie in den Bereichen Umwelt, Industrie, Pharmaindustrie, Landwirtschaft, Zahnmedizin und Medizin ein Umdenken eingesetzt hat. Es geht überall nicht mehr um einzelne Bakterienarten, sondern um Biofilme, auf Oberflächen räumlich organisierte Gemeinschaften von Mikroorganismen. Diese Tatsache stellt doch einiges auf den Kopf, das bisher und seit den Anfängen der mikrobiologischen Forschung so eindeutig nicht erkannt worden ist.

SWISS PHARMA: «Mehr als 30 Jahre im Gespräch mit der Pharmazeutischen Industrie der Schweiz» – Live-Interviews der Jahre 1979 bis 2011

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 34. Jahrgang (2012) 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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