Institut für Pharmazeutische Wissenschaften
Institute of Pharmaceutical Sciences

PhaWi Graz

Forschungs-Retreat und Doc Day des Instituts für Pharmazeutische Wissenschaften der Karl-Franzens-Universität Graz

6. – 7. Juli 2015

Einladung zum IPW Forschungs-Retreat und Doc Day

Nach drei PhaWi Graz Workshops 2006, 2007 und 2010, soll nun diese Reihe wieder aufgenommen werden. Aktueller Anlass ist die Berufung von zwei neuen Professoren im Bereich der Pharmazeutischen Wissenschaften, die sich bei dieser Gelegenheit vorstellen werden.

Gleichzeitig bietet sich aber die Gelegenheit, dass sich auch die vorhandenen Arbeitsgruppen präsentieren, so dass auch eine Leistungsschau des Instituts für unsere neuen Kollegen erfolgt.

Außerdem werden wir das Forschungs-Retreat auch als Doc Day nutzen. Alle unsere Dissertantinnen und Dissertanten sind herzlich eingeladen, in Kurzvorträgen oder Postern über Ihre Doktorarbeiten zu berichten.

Das Institut für Pharmazeutische Wissenschaften existiert nun seit über zehn Jahren. Durch die in den letzten Jahren erfolgten Veränderungen und auch durch die kürzlichen Neuberufungen ist es an der Zeit, den Forschungsschwerpunkt des IPW „*Cellular Stress and Tissue Dysfunction*“ neu zu überdenken, und für die Zukunft Überlegungen über mögliche Schwerpunkte und Kooperationen anzustellen.

Es sind daher alle wissenschaftlichen MitarbeiterInnen des IPW herzlich eingeladen, an diesem Forschungs-Retreat und Doc Day teilzunehmen, und sich ggf. aktiv mit einem Vortrag oder Poster einzubringen. Die Kosten für Unterbringung und Verpflegung werden übernommen.

Die entspannte Atmosphäre im Naturhotel Klugbauer am Reinischkogel wird dazu beitragen, dass wir uns noch besser kennenlernen, und dass auch zwischen den Vorträgen und am Abend ein reger Gedankenaustausch erfolgt.

Univ.-Prof. Dr. Rudolf Bauer
Leiter des Instituts für Pharmazeutische Wissenschaften

Programm

Montag, 6. Juli 2015

10:00 Begrüßung durch den Institutsleiter

10:15 Valery Bochkov
Complexity of cellular stress reactions induced by oxidized phospholipids

10:50 Robert Weis, Edith Größnitzer, Werner Seebacher, Antje Hüfner, Armin Presser, Johanna Faist
Synthesis and structure elucidation of bioactive compounds

11:15 Martin G. Schmid, Magdalena Taschwer, Jennifer Weiß, Stefan Mohr, Lucia Bodingbauer, Kian Kadkhodaei
Separation of recreational drugs by HPLC, GC and CE

11:40 Maximilian Aigner, Anita Jerkovic, Miriam Russ, Susanne Hauser, Philipp Pelzmann, Viktoria Müllner, Astrid Ortner
Electrochemical (Bio)Sensors in Food Control and/or as Diagnostic Tools

12:05 Andreas J. Kungl
Studying and therapeutically exploring chemokine-glycosaminoglycan interactions

12:30 Gemeinsames Mittagessen

14:00 Ulrich Stelzl
Human protein interaction networks in cellular signaling

14:45 Rudolf Bauer, Eva-Maria Pferschy Wenzig, Marlene Monschein, Sabine Ortmann
Application of plant metabolomics in medicinal plant research and drug discovery

15:10 Franz Bucar, Abraham Wube, Sandra Prasch, Olaf Kunert, Martin Waditzer, Ivana Turek, Antje Hüfner, Simon Gibbons, Sanjib Bhakta, Sonja Smole Mozina
Antibacterial and resistance modifying plant natural products

15:35 Kaffeepause

16:00 Kurt Schmidt, Bernd Kolesnik, Antonius C.F. Gorren, Bernd Mayer
Crosstalk between nitric oxide and hydrogen sulfide

16:25 Astrid Schrammel, Marion Mussbacher, Heike Stessel, Gerald Wölkart, Günther Haemmerle, Rudolf Zechner, Bernd Mayer
Role of adipose triglyceride lipase in cardiovascular (dys)function

16:50 Eva Roblegg
Drug Delivery across oral biological barriers

17:15 Andreas Zimmer
Protamine-oligonucleotide-nanoparticles: recent advances in drug delivery and drug targeting

17:40 Zusammenfassung (Rudolf Bauer)

19:00 Gemeinsames Abendessen

anschl. „Kamingespräche“

Dienstag, 7. Juli 2015

7:30 Gemeinsames Frühstück

8:30 Sara Crockett
Title to be announced

8:45 Christina Mauerhofer, Valery Bochkov
Novel mechanisms increasing endothelial stress tolerance: Insights from natural defense feedback and medicinal plants

9:00 Diogo G. Lopes, Karin Becker, Detlev Haack, Michael Stehr, Andreas Zimmer, Sharareh Salar-Behzadi
Combining hot-melt coating with lipid excipients and dry granulation for user-friendly solid oral dosage forms: the study case of ibuprofen sodium dihydrate

9:15 A-L. Schachner-Nedherer, Andreas Zimmer
Development of a nanoparticle-based miRNA delivery system to regulate adipocyte differentiation

9:30 Rupert Derler, Bernd Gesslbauer, Andreas J. Kungl
Semi-automatic characterization of glycosaminoglycan oligosaccharides by a combined mass spectrometry bioinformatics approach

9:45 Marissa Opelt, Matteo Beretta, Michael Russwurm, Doris Koesling, Astrid Schrammel, John Fassett, Bernd Mayer
Role of aldehyde dehydrogenase 2-catalyzed nitric oxide formation in nitroglycerin-induced vasorelaxation

10:00 Mohammed Noureldin, John Fassett, Christian L. Heine, Kurt Schmidt
Cellular type-specific recycling of tetrahydrobiopterin by dihydrofolate reductase

10:15 Kaffeepause und Postersession

1. Andrea Neubauer, Alexander Kollau, Regina Neubauer, Michael Russwurm, Doris Koesling, Astrid Schrammel, Bernd Mayer
Scavenging of nitric oxide in cytosols of porcine coronary arteries

2. Heine C.L., Schmidt R., Geckl K., Stuehr D.J., Schmidt K., Mayer B., Gorren A.C.F.
Selective Inhibition of the Neuronal and Inducible Isoforms of Nitric-Oxide Synthase by Hydrogen Sulfide

3. Tanja Gerlza, Sophie Winkler, Aid Atlic, Nikola Kitic, Roland Weis, Lubor Borsig, Andreas J. Kungl
Designing a mutant CCL2-HSA chimera with high glycosaminoglycan-binding affinity and selectivity

4. Marion Mussbacher, Heike Stessel, Gerald Wölkart, Günter Haemmerle, Rudolf Zechner, Bernd Mayer, Astrid Schrammel
Role of the ubiquitin-proteasome system in cardiac dysfunction of adipose triglyceride lipase deficient-mice

5. Nikola Kitic, Andreas J. Kungl
Applying size exclusion chromatography to study chemokine interactions

6. Werner Seebacher, Johanna Faist, Sarfraz Ahmad, Volker Wolkinger, Noor ul Amin Mohsin, Robert Weis
   New antiprotozoal compounds derived from bicyclic ring systems

7. Nikles Stefanie, Monschein Marlene, He Xiaojuan, Bian Yanqin, Lu Aiping, Min Yang, De-an Guo, Rudolf Bauer
   Metabolic Profiling of the Chinese Herbal Formula Bai Zhu Huang Qi Tang and its Immunomodulatory Effects in U937 Cells

8. Pan S, Bauer R
   Inhibition of COX-2 gene expression in LPS stimulated THP-1 macrophages by *Epipremnum pinnatum*

11:30 Zusammenfassung und Diskussion über Kooperationschancen und Möglichkeiten einer neuen Schwerpunktbildung (Moderation durch den Institutsleiter)

Ende ca. 13:00 Uhr

Check out und Abreise

Allgemeine Informationen

Veranstaltungsort: Der Klugbauer - Stefan Klug
Reinischkogel 28
8563 Reinischkogel
http://www.klugbauer.at/

Präsentationen: Die Vorträge werden in Englisch gehalten. Es sind bei jedem Vortrag 5 min für Diskussionen einzuplanen.
Die Poster sollten vor Beginn montiert werden. Während der Postersession solle der/die präsentierende Autor/in anwesend sein und für Fragen zur Verfügung stehen.

Unterbringung: Es sind Einzelzimmer und Doppelzimmer (DissertantInnen) reserviert

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Anreise: selbst zu organisieren
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Informationen zur Anreise: http://www.klugbauer.at/anfahrt.html

Auskünfte: Prof. Dr. Rudolf Bauer
Universitätsplatz 4
8010 Graz
Tel. 0316 / 380-8700
e-mail: pharm.wiss@uni-graz.at

Abstracts

Complexity of cellular stress reactions induced by oxidized phospholipids

Valery Bochkov
Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz

Enzymatic and non-enzymatic oxidation of free and esterified PUFAs is an important mechanism generating a broad spectrum of mediators demonstrating biological activities that were not characteristic of their unoxidized precursors. Oxidation of PUFAs esterified in phospholipids generates oxidized phospholipids (OxPLs) known to accumulate in atherosclerotic vessels. OxPLs induce multiple effects potentially relevant to atherosclerosis including proinflammatory and toxic action on endothelial cells. In good agreement with these pathological effects, OxPLs have been shown to activate cellular stress pathways such as unfolded protein response and electrophilic stress response. However, OxPLs also induce several biological effects that do not fit into the stereotypical action of „bad“ lipids. For example, exogenously applied OxPLs have been shown in vitro and in vivo to inhibit inflammation induced by agonists of toll-like receptors 2 and 4, protect endothelial barrier in lungs and induce angiogenesis. We made an unexpected observation that addition of OxPLs to cultured endothelial cells protected them from toxic action of low-serum medium, chemical inducers of apoptosis, lysophosphatidylcholine or hydrogen peroxide. Our data suggest that at least partially these effects were mediated by production of growth factors, peptides and eicosanoids acting in a paracrine manner. Cellular stress pathways played an important role in upregulation of protective mediators. These beneficial effects apparently represent a compensatory reaction of endothelial cells to the toxic action of OxPLs and thus demonstrate an example of a phenomenon often referred to as eustress or hormesis. Further study of protective feedback induced by atherogenic OxPLs can help in identification of new mechanisms of endothelial protection and potential drug targets promoting return of stressed endothelium to healthy functional state.

Synthesis and structure elucidation of bioactive compounds

Robert Weis, Edith Gößnitzer, Werner Seebacher, Antje Hüfner, Armin Presser, Johanna Faist
Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz

The members of the research group for drug development have gained experience in the synthesis and the structure elucidation of a wide variety of compounds. Heterocyclic ring systems have been prepared and functionalized. They were rearranged or fused to other ring systems giving compounds with antihistaminic, antimycobacterial or antiplasmodial activity [1-5]. A couple of natural compounds and their analogs were partially or totally synthesized, such as lignanes [6-8], terpenes [9,10], quinolones [3,4], and fatty acids [11]. Reactive carbohydrate building blocks have been prepared using orthogonal protecting group technology [12]. Terpenes have been functionalized and were reacted with these sugar moieties yielding mono-, di- and triglycosides as well as ortho esters with haemolytic properties [13]. Smart syntheses for bicyclic ring systems were developed. In the meanwhile several of their ring atoms have been functionalized giving rise to the synthesis of compounds that have far better antiplasmodial and antitrypanosomal activity than the parent compounds [14]. The newly synthesized compounds are characterized with the aid of 1- and 2 dimensional NMR techniques [1-14].

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Separation of recreational drugs by HPLC, GC and CE

Martin G. Schmid, Magdalena Taschwer, Jennifer Weiß, Stefan Mohr, Lucia Bodingbauer, Kian Kadkhodaei

Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz

In the past years, new amphetamines, cathinones and benzofuries have entered the global drug market being misused as “recreational drugs”. They cause serious social problems in many countries worldwide. Modification of their basic structure leads to a multitude of derivatives and all these “research chemicals” are chiral. As many chiral active pharmaceutical ingredients also the pharmacological effect of the enantiomers of those psychoactive compounds is supposed to differ.
In this work, a survey is given about different chromatographic and electrophoretic separation methods to obtain both achiral and chiral resolution of the afore mentioned compound classes.
First, capillary zone electrophoresis methods were developed for chiral separation of a broad spectrum of derivatives of amphetamines, cathinones and benzofuries by means of different chiral selectors [1-2]. Then, diverse HPLC methods are presented for separation of cathinones. For enantioseparation either a chiral selector can be immobilized onto the stationary phase [3] or added to the mobile phase [4]. Since cathinones are volatile they can be also separated by gas chromatography. An example for chiral separation after derivatization with a chiral reagent is shown [5].
Using optimal conditions most of the compounds were baseline resolved. It turned out that all samples purchased via the web consisted of racemic mixtures.

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Electrochemical (Bio)Sensors in Food Control and/or as Diagnostic Tool

Maximilian Aigner, Anita Jerkovic, Miriam Russ, Susanne Hauser, Philipp Pelzmann, Viktoria Müllner, Astrid Ortner

Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz

(Bio)Sensors are specific, rapid and economic devices and therefore interesting analytical systems for determining marker substances in food industry or clinical chemistry. Due to this fact bochkthe aim of the working group is to design and develop (bio)sensoric systems for the quantification of biogenic amines (cadaverine, putrescine, histamine, 2-phenylethlyamine, dopamine, norepinephrine) in biological matrix. The developed sensors could be valuable devices for disease diagnostics as well as for therapy control and for quality control of food samples.

The electrochemical biosensors are based on carbon paste working electrodes by detecting enzymatically produced hydrogen peroxide. To improve the performance of the sensor electrochemical mediators (e.g. MnO2) are added to the paste. Immobilization of the enzymatic bioreceptor (human monoamine oxidase B (hMAOB), pea seedling amine oxidase (PSAO)) is carried out in different ways to obtain good biocompatibility as well as good fixation of the protein on the surface of the electrode.

The optimized sensors were validated and tested for their applicability in biological matrix (e.g. fresh chicken meat) by using the standard addition method. The detailed analytics and the experiments in biological matrix make the developed biosensing FIA and/or bulk system a robust, sensitive and economic tool for the determination of biogenic amines in food samples. [1, 2]

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Studying and therapeutically exploring chemokine-glycosaminoglycan interactions

Andreas J. Kungl

Glycosaminoglycans (GAGs) are linear and highly charged (sulfated) polysaccharides which are found in the extracellular matrix of all human tissues as well as at the surface of every human cell. As part of proteoglycans, they modulate the activity of a plethora of proteins such as oncogenic growth factors, enzymes, chemokines, viral/bacterial proteins, etc. A database collection of the entire GAG-binding proteome is the framework for our research and a constant work in progress using conventional pull-down methods but also predictive bioinformatics as well as hand-curated literature search. In particular we are focusing on chemokine-GAG interactions which represent a therapeutically interesting but highly challenging interface for basic and applied research. We apply molecular modelling tools to infer structural implications of GAGs binding to chemokines, which enables us to rationally design a given chemokine with modified biological activity. In biophysical, cell-based and animal models we test the pharmacological activity of our engineered chemokines with the final aim of developing these biologic pro-drugs for clinical investigation. Our target indications include currently inflammatory lung disorders and metastatic cancer.

Human protein interaction networks in cellular signaling

Ulrich Stelzl

Max-Planck Institute for Molecular Genetics (MPIMG), Berlin
Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz

Comprehensive, high quality molecular interaction networks are prerequisite for a better understanding of cellular processes and associated phenotypes in health and disease and will foster drug development. Interaction networks are conditional with respect to the signaling status of the cell. The cellular response to internal and external cues is frequently mediated by the reversible covalent posttranslational modification (PTM), regulating protein activity, stability and protein-protein interactions (PPI). In combined experimental and computational approaches, we want to elucidate the role of post-translational protein modification for dynamic cellular processes.

We investigate whether different global post translational modifications, i.e. phosphorylation, acetylation and ubiquitination, are coordinated in human protein networks and how these PTMs are read by the cellular machinery. We identified hundreds of protein complexes that selectively accumulate different PTMs. Also protein regions of very high PTM densities, termed PTMi spots, were characterized and show domain-like features. The analysis of phosphorylation-dependent interactions provides clues on how these PPIs are dynamically and spatially constrained to separate simultaneously triggered growth signals which are often altered in oncogenic conditions. Our data indicate coordinated targeting of specific molecular functions via PTMs at different levels emphasizing a protein network approach as requisite to better understand modification impact on cellular signaling in health and disease.

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Application of plant metabolomics in medicinal plant research and drug discovery

Rudolf Bauer, Eva-Maria Pferschy Wenzig, Marlene Monschein, Sara Crockett, Sabine Ortmann,

Institute of Pharmaceutical Sciences, Department of Pharmacognosyy, University of Graz

Natural products possess a great structural diversity and consequently they still play a predominant role in the discovery of new drug leads [1]. However, searching for the active compounds in medicinal plants has been always like searching for the needle in the hay stack. Activity guided isolation has been a very effective but tedious approach to identify the active constituents. In recent years it has been complemented by *in-silico* screening and computer-aided methods [2]. Now, plant metabolomics based strategies are becoming increasingly important. LC-MS based metabolomics and multivariate data analyses allow correlations between the chemical profile of plants and the observed pharmacological activities, and to predict which compounds may contribute to the activity. LC-MS can also be used as a tool for dereplication.

The aim of a study funded within the NFN *Drugs from nature targeting inflammation* was to identify the anti-inflammatory active constituents of Chinese medicinal plant extracts by exploring the feasibilty of matching the UHPLC-HR-MS metabolite profiles with their *in-vitro* activity. Based on the results of a screening, the genera *Clematis* and *Lonicera* were chosen for this study. Ethanolic extracts from 48 Clematis and 36 Lonicera samples were included. The extracts were tested in several anti-inflammatory *in-vitro* assays by our collaborators in Vienna, and they were analyzed by UHPLC- ESI-HRMS in the negative mode at thed NAWI Graz Central Lab "Environmental, Plant & Microbial Metabolomics". The LC-MS data were processed in an untargeted approach by MZmine 2 [3]. Peaks were identified and quantified by Lipid Data Analyzer [4]. The abundance of the peaks was linked to the pharmacological activity of the extracts using SIMCA 13 [5]. Principal component analyses as well as orthogonal partial least squares–discriminant analyses (OPLS-DA) and visualization of the data by means of S-plot was conducted, which led to the identification of the compounds that were predicted to be most relevant for the *in vitro* anti-inflammatory activity in the particular bioassays. Finally the results need to be verified by testing the pure compounds. Isolation of minor compounds turned out to be a challenge. Nevertheless, this approach is a straight forward strategy for identification of active constituents which includes also synergistic effects.

Currently we are establishing a experimental platform for investigating the interaction of herbal extracts and human gut microbiota. Metabolic transformation of natural products by gut microbes on the one hand, and the influence of these compounds on gut microbiome on the other hand, shall be analyzed by using metabolomics and genomics techniques.

Acknowledgment:

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Antibacterial and resistance modifying plant natural products

Franz Bucar[a], Abraham Wube[a,b], Sandra Prasch[a], Olaf Kunert[b], Martin Waditzer[a], Ivana Turek[a], Antje Hüfner[b], Simon Gibbons[c], Sanjib Bhakta[d], Sonja Smole Mozina[e]

[a]*Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz,*
[b]*Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry; University of Graz*
[c]*UCL School of Pharmacy, Unviersity of London,*
[d]*Department of Biological Sciences, Birkbeck College, University of London,*
[e]*Biotechnical Faculty, University of Ljubljana*

During the last years therapeutic treatment of bacterial infections had to face the challenge of increasing incidence of antibiotic resistance [1] resulting in an urgent need for research into new antibiotic drugs with new modes of action. The aim of our current work is the isolation and characterization of antibacterials from various plant materials with emphasis on mycobacteria and *Campylobacter* spp. with different modes of action. These include efflux pump inhibitors (EPI), inhibitors of mycobacterial ATP dependent MurE ligase, inhibitors of the conjugal transfer of selected plasmids and inhibitors of bacterial adhesion. Aside from bioassay guided isolation strategies, synthesis of natural product derivatives is applied.
Our studies of *Alpinia katsumadai*, *Aframomum melegueta* and *Euodia rutaecarpa* revealed promising extracts and compounds like evocarpine derivatives, *trans,trans*-1,7-diphenylhepta-4,6-dien-3-one and [6]-paradol which were active as antimicrobials and modulators of antibiotic resistance in *Mycobacterium* and *Campylobacter* spp. [2-7]. Evocarpine analogues were found to be inhibitors of *M. tb.* MurE ligase [8].

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Crosstalk between nitric oxide and hydrogen sulfide

Kurt Schmidt, Bernd Kolesnik, Antonius C.F. Gorren, Bernd Mayer

Institute of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, University of Graz

Nitric oxide (NO) and hydrogen sulfide (H_2S) are signal molecules with regulatory roles in the cardiovascular, nervous, and immune systems. While some of the vascular actions of H_2S resemble those of NO - and are, at least partially, mediated by NO - there is evidence indicating that H_2S can also inhibit the formation and/or function of NO. In a project aimed at elucidating the mechanism underlying this negative cross talk, we observed that incubation of porcine aortic endothelial cells with the H_2S donors sodium hydrogen sulfide (NaHS) and GYY4137 blocks activation of endothelial NO synthase (eNOS) by the receptor agonist ATP but not by the Ca^{2+} ionophore A23187. Analysis of H_2S effects on Ca^{2+} dynamics revealed that H_2S inhibited ATP-induced release of Ca^{2+} from intracellular stores indicating that H_2S attenuates eNOS activity by blocking capacitative Ca^{2+} entry (1). In addition to its inhibitory effect on eNOS activation, H_2S was also found to impair the bioactivity by two distinct mechanisms. One involves inactivation of NO by superoxide arising from metal-catalyzed autoxidation, the second mechanism a direct reaction of NO with H_2S, yielding thionitrous acid (HSNO) In contrast to other reports claiming that HSNO may serve as a storage pool of NO (2,3) our data suggest that HSNO rapidly decomposes to bio-inactive nitrite.

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Role of adipose triglyceride lipase in cardiovascular (dys)function

Astrid Schrammel[a], Marion Mussbacher[a], Heike Stessel[a], Gerald Wölkart[a], Günther Haemmerle[b], Rudolf Zechner[b], Bernd Mayer[a]

[a]*Institute of Pharmaceutical Sciences, Department of Pharmacology, University of Graz*
[b]*Department of Molecular Biosciences, University of Graz*

Within the last decades, the prevalence of obesity and obesity-related cardiovascular diseases has been escalating world-wide due to the modern life style and nutritional behavior. Nowadays, adipose tissue is no longer regarded as passive store of excess energy but considered a highly active endocrine organ that secretes various humoral factors. Coincidence of obesity, insulin resistance or overt type 2 diabetes, dyslipidaemia (and hypertension) is clinically termed metabolic syndrome. In view of the devastating medical consequences of obesity, the development of novel therapeutic strategies is pivotal and necessitates the availability of appropriate models.

Mice lacking adipose triglyceride lipase (ATGL), a key enzyme of mammalian lipolysis represent the rodent correlate to human neutral lipid storage disease with myopathy [1]. The murine phenotype is characterized by massive accumulation of neutral lipids in multiple adipose and non-adipose tissues and organs. In the heart, ectopic accumulation of triglycerides results in the progressive development of lethal cardiomyopathy. Due to the unique metabolic profile (increased insulin sensitivity and glucose tolerance) ATGL knockout mice are an excellent tool to study obesity-mediated cardiovascular phenomena without interference of comorbidities of the metabolic syndrome.

In this talk a brief overview of the scientific background, study aims, methodical approaches and results on the role of ATGL in cardiovascular (dys)function will be presented.

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Drug Delivery across oral biological barriers

Eva Roblegg

Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, University of Graz

Drug delivery and drug targeting are disciplines in the field of pharmaceutical technology that aim at facilitating the transport of critical active molecules to their site of action, at appropriate times, in a controlled manner. Currently, one potential area in drug delivery is the tailoring of nanocarriers, which protect the drug from enzymatic degradation and are small enough to cross biological barriers. Thereby, the oral administration route, which is still the most common and convenient route, represents an interesting, but very challenging field. As a consequence, alternative routes for drug administration - such as the oral cavity - that avoid hepatic first pass metabolism are of enormous interest. Since permeability of molecularly dissolved drugs is likely not to be comparable with the permeability behavior of nanoparticles (NPs), fundamental knowledge regarding the main barriers that impact particle uptake is of enormous interest [e.g.,1,2]. At the same time, possible cytotoxic effects have to be considered, since several studies suggest that NPs may cause injuries to biological systems [e.g., 3,4]. To study this in detail, modeling of biological barriers by *in vitro* cell cultivation has received increased attention. One of the major advantages of such *in vitro* models is that cellular and sub-cellular functions can be studied in complex systems, achieved by co- and triple-cultivation of different human cell lines [e.g, 5,6,7].
These models also serve the purpose to gain deeper mechanistic insights in alcohol-induced dose dumping effects of controlled-release dosage forms [e.g., 8, 9]. Recently, this phenomenon has drawn significant attention of the regulatory authorities (FDA, EMA), since the concomitant intake of alcoholic beverages together with oral controlled-release formulations, comprising highly potent drugs with a narrow therapeutic window poses a serious safety concern. Alcohol has the potential to alter the release rate controlling mechanisms of the dosage form resulting in an unintended, rapid release of the drug in a short period of time. So far, only a limited number of dosage forms that withstand the impact of alcohol are available. Hence, for the design of a robust and alcohol-resistant dosage form, it is vital to systematically analyze the physico-chemical key factors of the drug, the excipients and the properties of the final dosage form, considering the gastrointestinal physiology.

For practical application of (nano) drug delivery systems as well as alcohol resistant formulations, the pharmaceutical industry requires new, safe and efficient strategies that combine existing conventional know how with innovative drug delivery knowledge and novel processing methods. Thus, new technologies (such as hot melt extrusion and wet extrusion) are required that enable a continuous transformation of drug loaded (nano)carriers into a solid formulation [e.g.,10,11]. This allows to (i) precisely control the product quality, (ii) increase the manufacturing capacity, and (iii) to produce a range of drug doses in an efficient manufacturing environment.

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Protamine – oligonucleotide – nanoparticles: recent advances in drug delivery and drug targeting

Andreas Zimmer

Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, University of Graz

In the year 2000 for the first time a new method for preparing solid nanoparticles from antisense oligonucleoides together with the cationic peptide protamine was invented by our group. Before, the aggregation into compact structures with short segments of single-stranded DNA was only reported with the polycation poly(L-lysine). We have named this new drug delivery system “proticles” [1]. Within our next study comparing the polycations protamine, spermine and spermidine in terms of their potential to condense different types of oligonucleotides and antisense drugs, protamine was found to be most efficient to form nanoparticles in the size range of 100 - 200 nm [2]. Protamine a peptide well known as pharmaceutical excipient is derived from the sperm of salmon with a molecular mass of approx. 4000 Da and consists of about 70 mol% arginine. In our first evaluations as colloidal nanosuspension these nanoparticles protected oligonucleotides very efficiently against enzymatic digestion caused by nucleases. Also the very early research in this field demonstrated an improved cellular uptake of oligonucleotides combined with significant antisense effects in-vitro [2].

Antisense drugs in general were evaluated as potential anti-viral substances starting from 1990 and in this field of research we could demonstrate for proticles loaded with an AS-PTO drug directed against human immunodeficiency virus type 1 (HIV-1) tat mRNA a very efficient transfection of HIV-1 target cells. Protamine was used to complex AS-ODN and AS-PTO to form nanoparticles with diameters of about 180 nm and surface charges up to +30 mV. Cellular uptake of these nanoparticles was significantly enhanced compared to naked oligonucleotides and showed the release of the antisense compound leading to a specific inhibition of tat mediated HIV-1 transactivation [3]. Further research in this field characterized the physicochemical properties of these new nanoparticles and a comparison between different transfection reagents showed lowest cytotoxicity in-vitro for proticles but highest efficacy for cationic lipids [4,5]. Therefore next steps in research included further optimization of the protamine nanoparticles. Albumin was found to be a potent stabilizer of proticles and in addition to the basic binary systems, ternary systems showed superior properties in terms of cellular uptake and intracellular ODN distribution [6-8]. A combination of proticles with liposomes was reported in 2005. Junghans et al. showed the possibility to coat the protamine ODN particle with a lipid film. Further innovation came from the application of protamine sulfate to modify the particle diameter in the lower nanometer range more efficiently [9,10]. One year later the first publication which evaluated the immunogenic properties of proticles showed also the possibility to improve the immune-modulation of CpG oligonucleotides which were loaded into proticles [11]. Most successfully proticles with non-immunogenic CpG control oligonucleotides were found to be not immunogenic at all and all basic proticle formulation including pure protamine were tolerated very well and were also found to be highly biocompatible in-vivo.

Therefore the next steps in research addressed the question of activ drug targeting by coating the nanoparticle with targeting sequences or to use the proticles as depot system for peptide drugs [12,13]. Consequently in 2010 it was demonstrated for the first time to target proticles loaded with VIP

specifically to tumor cells which overexpressed the VPAC receptor. This approach could be also demonstrated in human lung tumor tissue ex-vivo [14].

Further, targeting of proticles was established for diagnostic purposes using adiponectin as targeting sequence for enhanced imaging of atherosclerotic plaques [15]. More recently our research included also methods to simulate the self-assembly process which is responsible for the nanoparticle formation and to establish a microreactor technology to scale-up the manufacturing process [16-17]. Up to now, this improved technology again was applied to study the possibility of proticle to act as adjuvant and immune-modulator in-vivo [18] and in combination with an improved IL-10 mediated targeting differences were investigated between proticles and targeted liposomes ex-vivo in mice [19].

Natural Products with Antibacterial and Anti-Inflammatory Bioactivity from *Hypericum* (St. John's wort, Hypericaceae)

Sara L. Crockett

Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz

I have conducted research on the phytochemistry of *Hypericum* (Hypericaceae), a flowering plant genus with ca. 490 species, one of which is St John's wort (*H. perforatum* L.), a medicinal crop plant with economic importance due to its anti-viral, anti-bacterial, and anti-depressive properties, over the past 15 years. This research has resulted in the isolation and identification of natural products with bioactivity against neurodegenerative disease targets and infectious microorganisms and fits well within one of the seven focal research areas for the University of Graz, "Molecular Enzymology and Physiology", which has a specific focus on neurodegenerative diseases in the context of aging research and molecular bacteriology and infection biology. *Hypericum* species produce a rich diversity of bioactive plant secondary metabolites, in particular derived from polyketide biosynthetic pathways, such as flavonoids, biphenyls, dibenzofurans, xanthones, and benzophenones. In the plant, these classes of compounds can serve as defense molecules, elicited in response to microbial infection, and often possess antibacterial activity against human pathogens[1-2]. Comprehensive research on the anti-depressive activity of *H. perforatum* extracts has shown that the synergistic properties of several classes of compounds, including naphthodianthrone derivatives (e.g., hypericins), flavonol glycosides, and acylphloroglucinol derivatives (e.g., hyperforin) are crucial[3].

The interdisciplinary research conducted has included cultivation trials, genetic and phytochemical profiling, chemical ecology investigations, and essential oil analyses of targeted species, as well as isolation and structural elucidation of secondary metabolites with anti-depressive, antibacterial, and anti-inflammatory bioactivity. As examples, results of two projects are presented. First, bioassay-guided fractionation of *H. perforatum* root extracts, testing for growth inhibition of plant pathogenic fungi from the genera *Colletotrichum*, *Botrytis*, *Fusarium* and *Phomopsis*, as well as *in vitro* anti-inflammatory activity through inhibition of COX-1, COX-2 and 5-LOX-catalyzed LTB_4 formation, resulted in the identification of xanthones that displayed novel bioactivity against species of *Phomopsis* and inhibited 5-LOX-mediated LTB_4 formation. Second, the *in vitro* inhibitory potential of 50 extracts from various *Hypericum* species against *Paenibacillus larvae*, a spore-forming, Gram-positive bacterial pathogen that causes American foulbrood (AFB), a lethal disease currently affecting honeybee brood worldwide was tested. 14 highly active extracts were targeted, and six bioactive acylphloroglucinol and filicinic-acid derivatives (MICs 0.168-220 µM) were isolated[4].

Implications of these findings are described and future research directions are discussed.

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Novel mechanisms increasing endothelial stress tolerance: Insights from natural defense feedback and medicinal plants

Christina Mauerhofer, Valery Bochkov
Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz

Endothelial stress and dysfunction represent the major events initiating atherosclerosis [1], which is the cause of more than half of deaths in western society [2]. This justifies the need of analysis of endogenous mechanisms of endothelial stress tolerance.

Many risk factors like smoking, diabetes [3] or hypertension [4] are accompanied by generation of atherogenic oxidized lipids. In particular, phospholipids containing oxidized fatty acids (oxidized phospholipids, OxPLs) are thought to play important role as culprits of atherosclerosis [2]. Although the proinflammatory and toxic action of OxPLs is well documented, a recent observation made in Prof. Bochkov's group showed a protective effect of OxPLs on endothelium at low concentrations [5, unpublished data]. This leads to the hypothesis, that protective action might be a compensatory reaction against the toxicity of OxPLs. The tolerance to OxPLs at least partially was mediated by soluble proteins and peptides, some of which were identified by the group of Prof. Bochkov. These protein mediators are already known to protect endothelial cells and support endothelial regeneration [unpublished data].

The aim of this PhD thesis is to identify further soluble and endothelium protective mediators induced by OxPLs and characterize their mechanism of action in respect to inhibition of apoptosis, stimulation of endothelial proliferation and cell migration, as well as recruitment and differentiation of endothelial precursor cells. Moreover the mechanisms underlying upregulation of these proteins will be investigated. For this reason, transcriptional regulation of endothelium-protective genes will be analyzed. In summary, this project is focused on the investigation of basic mechanisms of endothelial stress tolerance. Insights in this field will lead to an improved understanding of the pathogenesis of a variety of diseases being linked to endothelial dysfunction and cell damage.

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Combining hot-melt coating with lipid excipients and dry granulation for user-friendly solid oral dosage forms: the study case of ibuprofen sodium dihydrate

Diogo G. Lopes[a], Karin Becker[b], Detlev Haack[c], Michael Stehr[d], Andreas Zimmer[b], Sharareh Salar-Behzadi[a]
[a]*Research Center Pharmaceutical Engineering (RCPE) GmbH, Graz*
[b]*Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, University of Graz*
[c]*HERMES ARZNEIMITTEL GmbH, Großhesselohe, Germany*
[d]*CREMER OLEO GmbH & Co. KG, Witten, Germany.*

The simple and effective orally delivery of an active pharmaceutical ingredient (API) is an important factor influencing treatment efficacy, tolerability and patient compliance. To further improve the user-friendliness and acceptance of solid oral dosage forms, multiparticulate systems are becoming more common. As they have proven especially popular with patients requiring regular doses or those that find it difficult to swallow solid tablets, such as children, the elderly or individuals suffering from esophagitis. However, for this method to prove effective, most APIs must be effectively coated. Traditional methods to coat solid oral dosage forms mostly use solvents, an approach that suffers from a range of drawbacks including environmental concerns, lengthy processing times and the risk of dissolving drug. Hot melt coating (HMC) is an innovative and effective alternative that is reliable, cheap and fast, while simultaneously offering an opportunity to better manipulate and control characteristics such as taste, stability and release rate.

In this work, the study case of ibuprofen sodium dihydrate is presented. This chemical variation of ibuprofen, a medicine currently authorized in adults and children for various indications, as shown faster onset of action in pain and fever treatment. However, the sodium salt is known as exceptionally poorly compressible and tablet formulations need high quantities of compressible fillers and disintegrants in order to obtain, nevertheless, useful compression and disintegration properties resulting in difficult to swallow large tablets. Dry granulation formulation and process were developed to produce high drug loaded cores (94%) of ibuprofen sodium dihydrate suitable for coating process. These cores were taste masked via hot-melt coating resulting in easy to swallow and fast onset of action dosage form.

Development of a nanoparticle-based miRNA delivery system to regulate adipocyte differentiation

A-L. Schachner-Nedherer, A. Zimmer
Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, University of Graz

MicroRNAs (miRNAs) are a class of non-protein coding small RNA molecules of approximately 22 nucleotides. The miRNA system is an endogenous mechanism which regulates gene expression at posttranscriptional level by specific mRNA binding. Recent investigations indicate that these tiny molecules play an important role in different biological processes like cell proliferation, differentiation and apoptosis and exhibit tissue-specific expression patterns [1, 2].

Obesity and the metabolic syndrome are public health concerns and require an improved understanding of its related metabolic disorders to develop new therapeutic strategies. The complex process of adipocyte differentiation is controlled by various transcription factors such as peroxisome proliferator-activated receptor gamma (PPARγ) which represents a master transcriptional factor [3-5].

The intention of this PhD thesis is the development of a nanoparticle-based delivery system composed of miRNA-27a and protamine for adipocyte transfection. miRNA-27a is known to inhibit adipocyte differentiation by suppressing PPARγ [6, 7]. The basic investigations address the establishment of an efficient differentiation and transfection protocol in vitro using murine cell lines named 3T3-L1 and 3T3-F442A. Commercial available transfection reagents such as HiPerFect are complexed with miRNA-27a and serve as positive control for the nanoparticle-based drug delivery system. The formation of these nanoparticles is based on electrostatic interactions between the arginine rich peptide and the negatively charged nucleic acid. The carrier system should be capable of protecting miRNAs against enzymatic digestion caused by nucleases and promoting cellular uptake and distribution. Transfection efficiency is evaluated by using a fluorescent labelled transfection control and the extent of adipogenesis in mature adipocytes is visualized by Oil Red O staining. Further investigations comprise the expression pattern of miRNA-27a during adipogenesis and its effect on target mRNAs. In addition, characterization of the miRNA-loaded nanoparticles including particle size and zeta potential is performed.

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Semi-automatic characterization of glycosaminoglycan oligosaccharides by a combined mass spectrometry bioinformatics approach.

Rupert Derler[ab], Bernd Gesslbauer[a], Andreas J. Kungl[ab].

[a]*Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz*
[b]*Antagonis Biotherapeutics GmbH, Graz.*

Glycosaminoglycans (GAG) are negatively charged polysaccharides expressed by almost all animal cells. They play important roles in physiological and pathophysiological events like inflammation, cell differentiation, cancer, neurodegeneration and infectious diseases. There may well be more than 1000 proteins that interact with GAGs. The selectivity and specificity of binding depends on GAG chain modifications like O- and N-sulfation, N-acetylation and epimerization. Therefore, characterization of GAG chains is needed to therapeutically target these glycans [1]. So far, there is no method available to sequence naturally occurring GAGs. The combination of mass spectrometry and bioinformatics tools like GlycoWorkBench [2] can largely facilitate characterization and identification of heparin/heparan sulfate oligosaccharides by reducing the amount of manual data interpretation.

For this approach, size-defined GAG oligosaccharides were diluted to a final concentration of approx. 10 pmol/µl and analyzed by mass spectrometry as previously described by *Köstner et al*. [3] on a LCQ Deca XP^+ system (Thermo Fisher). After a full MS^1 scan, the most abundant peaks were isolated for determination of charge and subsequent fragmentation (MS^2). A multi-step, semi-

automatic approach was established that makes use of MS^1-, MS^2- and isolated peak-data to determine the structure of GAGs.

This method allows for identification of differentially modified synthetic and natural GAG tetra-saccharides. Furthermore, it is possible to determine the degree of sulfation and acetylation in mixtures of size-defined heparin/heparan sulfate GAGs with a polymerization grade between two and six saccharides.

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Role of aldehyde dehydrogenase 2-catalyzed nitric oxide formation in nitroglycerin-induced vasorelaxation

Marissa Opelt[a], Matteo Beretta[b], Michael Russwurm[c], Doris Koesling[c], Astrid Schrammel[a], John Fassett[a] and Bernd Mayer[a]

[a]*Institute of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, University of Graz*
[b]*King's College London, British Heart Foundation Centre of Research Excellence, Cardiovascular Division, UK*
[c]*Department of Pharmacology and Toxicology, Ruhr-Universität Bochum, Germany*

Nitroglycerin (GTN) bioactivation in vascular smooth muscle to nitric oxide (NO) or a related species activates soluble guanylate cyclase (sGC), and results in cGMP-mediated vasodilation. In 2002, Stamler and coworkers identified aldehyde dehydrogenase-2 (ALDH2) as the pivotal enzyme responsible for GTN bioactivation and proposed that ALDH2 catalyzes bioconversion of GTN to yield 1,2-glyceryl dinitrate (1,2-GDN) and nitrite. This reaction appears to be associated with formation of a disulfide between the catalytic Cys302 residue and one of the vicinal cysteines (Cys301 or Cys303). Reduction of nitrite to NO by components of the mitochondrial respiratory chain was suggested as link between ALDH2-catalyzed GTN metabolism and vasorelaxation [1]. However, this view was challenged by the findings that GTN activates sGC in the presence of purified ALDH2 and GTN biotransformation is not affected by substrates and inhibitors of the respiratory chain [2]. Previous studies with a purified ALDH2 mutant lacking the cysteine residues adjacent to the active Cys302 site (C301S/303S mutant) improved sGC activation and showed increased NO formation compared to wild-type ALDH2. These findings showed that ALDH2 is able to catalyze direct reduction of GTN to NO [3].

In this project, we aim to clarify the relevance of ALDH2-catalyzed NO formation in GTN-induced relaxation in blood vessels. In preliminary studies, wild-type ALDH2 and the C301S/303S mutant were overexpressed in murine ALDH2-deficient aortic smooth muscle cells. We observed that the C301S/303S mutant activates sGC more effectively in cell lysates than wild-type ALDH2, resulting in a decrease of the EC50 value from 2.87 ± 0.68 to 0.11 ± 0.01 µM in the presence of wild-type ALDH2 and mutant, respectively. ALDH2-catalyzed GTN denitration was reduced in the presence of the cysteine mutant, confirming that ALDH2-catalyzed NO formation can be distinguished from GTN denitration. Further studies with isolated ALDH2-deficient mouse aortas should shed light on the role of ALDH2-catalyzed NO formation in GTN-induced relaxation.

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Cellular type-specific recycling of tetrahydrobiopterin by dihydrofolate reductase

Mohammed Noureldin, John Fassett, Christian L. Heine, Kurt Schmidt

Institute of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, University of Graz

Dihydrofolate reductase (DHFR), an enzyme that catalyzes the reduction of dihydrofolate to tetrahydrofolate, also accepts dihydrobiopterin (BH2) as substrate and converts it to tetrahydrobiopterin (BH4), an essential cofactor of nitric oxide synthase. Based on data showing that the latter reaction is very slow in human cells, it had been questioned whether this pathway may indeed play a role in BH4 regeneration [1]. Using endothelial cells isolated from human umbilical vein and porcine aortic endothelial cells, we recently demonstrated that recycling of BH4 by DHFR is substantially more efficient in porcine than human cells, presumably due to a high binding affinity of BH2 to porcine endothelial DHFR [2] . To test whether this phenomenon is a peculiarity of endothelial cells or species related, we determined DHFR kinetics in cytosolic fraction of porcine, bovine and rat liver. The results revealed that the K_M of DHFR to BH2 in porcine liver was 5 ± 3 µM but 50 ± 5 µM and 25 ± 10 µM in liver of rat and cattle, respectively. Based on these data suggesting that the high affinity binding may be an intrinsic characteristics of porcine DHFR, we expressed both porcine and human DHFR in E. coli and purified the proteins by using affinity chromatography. Enzymatic characterization of the recombinant enzymes is currently performed.

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Scavenging of nitric oxide in cytosols of porcine coronary arteries

Andrea Neubauer[a], Alexander Kollau[a], Regina Neubauer[a], Michael Russwurm[b], Doris Koesling[b], Astrid Schrammel[a] and Bernd Mayer[a]

[a]*Institute of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, University of Graz*
[b]*Department of Pharmacology and Toxicology, Ruhr-Universität Bochum, Germany*

Bioactivation of nitroglycerin (GTN) yields nitric oxide (NO) or a related activator of soluble guanylate cyclase (sGC), resulting in cGMP formation and subsequent vasorelaxation [1]. In isolated vessels, effects of GTN are routinely measured as vasorelaxation in organ bath studies. However, the relaxant effect of GTN could not be reproduced in blood vessel homogenates using activation of purified sGC as *in vitro* assay of GTN bioactivation. In the present study, we investigated this discrepancy using porcine coronary arteries (CA) and aortas with the following methods: activation of purified sGC with the NO donor DEA/NO was measured in the presence of cytosolic preparations of CA and aortas under various conditions. NO consumption by cytosols was determined using a NO-sensitive electrode. UV/VIS spectroscopy was performed to test for the oxidation state of cytosolic hemeproteins.

Activation of sGC by 0.1 µM DEA/NO was almost abolished in presence of untreated cytosols but restored after pre-treatment with oxidants (hexacyanoferrate (III) or ODQ). NO consumption by the cytosolic preparations was protein-dependent and partially restored by treatment with the oxidant ODQ. UV/VIS spectroscopy revealed a characteristic heme-like oxidation-sensitive absorption spectrum of the cytosols. The NO scavenging effect was significantly more pronounced in the tunica media (smooth muscle) as compared to the intima (endothelium).

Our data suggest that a heme-containing protein is involved in scavenging of NO in cytosolic preparations of CA. The extent of NO consumption was in excellent correlation with the heme-content of the preparations. Further work towards identification and physiological functions of the scavenging protein is underway in our laboratory.

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Selective Inhibition of the Neuronal and Inducible Isoforms of Nitric-Oxide Synthase by Hydrogen Sulfide

Heine C.L.[1], Schmidt R.[1], Geckl K.[1], Stuehr D.J.[2], Schmidt K.[1], Mayer B.[1], and Gorren A.C.F.[1]
[1]*Institute of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, University of Graz*
[2]*Department of Pathobiology, Lerner Research Institute, The Cleveland Clinic, Cleveland (USA)*

Citrulline formation of both human neuronal nitric-oxide synthase (nNOS) and mouse macrophage inducible nitric-oxide synthase (iNOS) were inhibited by the hydrogen sulfide (H2S) donor Na2S with IC50 of 2.0±0.3•10–5 M and 5.9±0.7•10–5 M, respectively. In contrast, maximal specific activity of human endothelial nitric-oxide synthase (eNOS) was only decreased to ~ 80 % at 3 mM Na2S. Excess of cofactors did not alter IC50 for nNOS significantly, suggesting a non-competitive way of inhibition for H2S. Furthermore, we obtained a leftward shift of the IC50 to 1.4±0.3•10–5 M at pH 6.0 and a rightward shift of the IC50 to 6.4±0.3•10–5 M at pH 8.0, indicating that inhibition is induced presumably due to interaction of H2S with nNOS, rather than with HS-. Dilution experiments imply that inhibition of nNOS by H2S is irreversible, but only in the presence of nitric oxide synthesis. This was confirmed by the fact that NADPH oxidation of nNOS was only inhibited with similar IC50 as for citrulline formation inhibition in the presence of nitric oxide. Low-temperature gel electrophoresis suggests (LT-PAGE) and subsequent Western blot suggest that H2S in combination with nitric oxide has only a small impact on quaternary structure of eNOS, while dimeric strength of nNOS was significantly weakened. Our data demonstrate that nNOS and iNOS were selective inhibited by H2S/NO, maybe due to interaction with the dimer interface, whereas eNOS where scarcely affected.

Designing a mutant CCL2-HSA chimera with high glycosaminoglycan-binding affinity and selectivity

Tanja Gerlza[a,d], Sophie Winkler[a], Aid Atlic[b], Nikola Kitic[a], Roland Weis[b], Lubor Borsig[c] and Andreas J. Kungl[a,d]
[a]*Institute of Pharmaceutical Sciences, Department of Pharmaceutiocal Chemistry, University of Graz*
[b]*VTU Technology, Grambach, Austria*
[c]*Institute of Physiology, University of Zürich and Zürich Center for Integrative Human Physiology, Switzerland*
[d]*Antagonis Biotherapeutics, Graz*

The CC- chemokine MCP-1 plays a crucial role in many acute and chronic inflammatory diseases by mediating leukocyte migration to inflammatory sites by binding to G-protein coupled receptors on the one hand and to glycosaminoglycans (GAGs) on the endothelium of the inflamed tissue on the other hand [1]. The CCL2-CCR2 chemokine axis has also been identified to be involved in cancer progression where it contributes to metastatic dissemination, through modulating both tumor cell metastatic behavior and the metastatic microenvironment [2]. We have designed an MCP-1-based decoy protein (PA910) which shows increased binding affinity to specific GAG structures combined with a knock-out of the chemokine's GPCR activity [3]. This dominant-negative mutant MCP-1 (dnCCL2) was shown to be highly bio-active in several animal models of inflammatory diseases. To extend its serum half-life and to improve its bioavailability *in vivo*, dnCCL2 was fused to human serum albumin (HSA).

We have analyzed GAG binding affinities, stability/unfolding, secondary structure, oligomerisation pattern, serum half-life and the selective chemokine displacement of this dnCCL2-HSA chimera in comparison to the unfused decoy and to the wild type protein. We found that this fusion did not lead to a loss in GAG-binding affinity, but significantly improved stability and bio-availability *in vivo*. Furthermore we could show with our ELICO method [4] that this HSA-fusion mutant displaces less other chemokines from heparan sulfate compared to dnCCL2 therefore being much more target selective.

The dnCCL2-HSA mutant was investigated in a human tumor cell migration model in mice, where the protein was found to bind effectively to the vasculature and to block tumor cell seeding in the lung. Therefore this fusion protein represents a promising therapeutic approach to attenuate metastasis.

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Role of the ubiquitin-proteasome system in cardiac dysfunction of adipose triglyceride lipase deficient-mice

Marion Mussbacher[a], Heike Stessel[a], Gerald Wölkart[a], Günter Haemmerle[b], Rudolf Zechner[b], Bernd Mayer[a], Astrid Schrammel[a]

[a]*Institute of Pharmaceutical Sciences, Department of Pharmacology and Toxicology, University of Graz*
[b]*Department of Molecular Biosciences, University of Graz*

Adipose triglyceride lipase (ATGL) represents a key enzyme of the lipolytic cascade. Global ATGL deficiency in mice leads to massive accumulation of neutral lipids in adipose and multiple non-adipose tissues. In hearts of ATGL knockout mice, ectopic storage of triglycerides results in progressive development of lethal cardiomyopathy. Recently it was demonstrated that ATGL knockout mice suffer from pronounced cardiac oxidative inflammatory stress and defective PPARα signaling.

Since dysfunction of the ubiquitin proteasome system (UPS) has been closely linked to various cardiac pathologies, we investigated if disturbances in cellular protein degradation might contribute to the observed cardiac phenotype. Western blot analysis revealed significantly increased amounts of ubiquitinated cardiac proteins in ATGL-deficient hearts. In parallel, protein expression of the ubiquitin-activating enzyme E1a, which initiates protein ubiquitination, was significantly upregulated in cardiac ATGL deficiency. Both effects were reversed upon cardiomyocyte-directed overexpression of ATGL in ATGL knockout mice.

In parallel, we observed activation of cardiac NF-κB signaling in those hearts. Chronic treatment of ATGL knockout mice with the PPARα agonist Wy14,643 (which substantially improves cardiac performance) reversed accumulation of ubiquitinated proteins, prevented activation of NFκ-B, and decreased oxidative stress.

In summary, our data suggest a hitherto unrecognized link between proteasomal function, PPARα signaling and cardiovascular disease.

Applying size exclusion chromatography to study chemokine interactions

Nikola Kitic, Andreas J. Kungl

Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz

Size exclusion chromatography (SEC) is a very important and widely used tool in protein biochemistry for analytical and preparative purposes. It can, for example, be used to determine the aggregate/oligomerisation size and content of pharmaceutical proteins [1], for interaction studies [2], or as a part of a manufacturing process [3].

We are applying SEC mainly to characterize the oligomeric state of chemokines and chemokine mutants, as well as of chemokine-glycosaminoglycan (GAG) complexes (4). The biology and pathology of chemokines is crucially dependent upon interactions with themselves – forming homo- or hetero-oligomers – a process which is strongly influenced by the interaction with GAGs. Therefore, engineering chemokines with respect to their GAG binding and oligomerisation behavior is a major tool to investigate the molecular biology of chemokines which itself can be studied by SEC. In a preparative sense, we are using SEC for the fractionation of glycosaminoglycan oligosaccharides which can further be used in various interactions studies as well as for chemokine target investigations.

Recent results of our studies will be presented and discussed.

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New antiprotozoal compounds derived from bicyclic ring systems

Werner Seebacher[a], Johanna Faist[a], Sarfraz Ahmad[b], Volker Wolkinger[c], Noor ul Amin Mohsin[a], Robert Weis[a]

[a]*Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, University of Graz*
[b]*Center of Research in Molecular Medicine, The University of Lahore, Pakistan*
[c]*Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz*

New antiprotozoal drugs, especially compounds with activity against Plasmodium falciparum (P.f.) and Trypanosoma brucei (T.b.), are urgently needed because these causative organisms of Malaria tropica and sleeping sickness show already resistance against drugs in use. We developed a simple synthesis for 4-dialkylamino-6,7-diphenylbicyclo[2.2.2]octanones from the reaction of unsaturated ketones and thiocyanates of secondary amines and reduced them to bicyclo[2.2.2]octan-2-ols. In a screening against some causative organisms of tropical diseases their antiplasmodial and antitrypanosomal activities were detected. Various modifications were examined using those lead compounds. An important optimization was the introduction of an additional nitrogen atom to the bicyclic skeleton. This was done by a Beckmann procedure with subsequent reduction giving 2-azabicyclo[3.2.2]nonanes. Some derivatives, bis-chloro compounds showed in vitro very promising activity against T.b. rhodesiense (IC_{50}: 0.06 µM). A second path was taken by use of a Schmidt reaction which ends up in 3-azabicyclo[3.2.2]nonanes. One of those products showed in vivo antimalarial activity in Peter's mouse model. This was the reason for a patent application in 2009. Since then, following modifications were done: The structure of the exocyclic amino residue in position 5 was varied. Concerning the two aromatic rings we added different substituents to both and even to only one ring to investigate the influence of electron density on the biological activity. Furthermore, we introduced several basic side chains to the ring nitrogen in position 3. One of those derivatives (IC_{50}: 0.022 µM) was in vitro more active against the multiresistant K_1 strain of P.f. than artemisinin (IC_{50}: 0.064 µM), but showed unfortunately no in vivo potency.

Metabolic Profiling of the Chinese Herbal Formula Bai Zhu Huang Qi Tang and its Immunomodulatory Effects in U937 Cells

Nikles Stefanie[a], Monschein Marlene[a], He Xiaojuan[b], Bian Yanqin[b], Lu Aiping[c], Min Yang[d], De-an Guo[d], Rudolf Bauer[a]

[a]*Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz*
[b]*China Academy of Chinese Medical Sciences, Institute of Basic Research in Clinical Medicine, Beijing, China;*
[c]*School of Chinese Medicine, Hong Kong Baptist University*
[d]*Shanghai Research Center for Modernization of Traditional Chinese Medicine, National Engineering Laboratory for TCM Standardization Technology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China.*

The classical TCM formula Bai Zhu Huang Qi Tang (BZHQT) is a popular mixture used traditionally for the treatment of ulcerative colitis. It consists of three herbs: Glycyrrhizae radix (Gan Cao), Astragali radix (Huang Qi) and Atractylodis macrocephalae rhizoma (Bai Zhu). TLC- and LC-DAD-MS/MS methods have been developed for the analysis of the metabolic profiles of BZHQT and the three single herbs. Decoctions of the single herbs and of the formula have been fractionated with *n*-hexane, dichloromethane, ethylacetate and *n*-butanol and have been analysed by TLC and HPLC in order to trace Glycyrrhizae radix, Astragali radix and Atractylodis macrocephalae rhizoma in BZHQT.
As a result, twenty-eight constituents of Glycyrrhizae radix, seven constituents of Astragali radix and three constituents of Atractylodis macrocephalae rhizoma have been assigned in the chemical profiles of the formula, which now allows a standardization of BZHQT.
The results of the pharmacological testing showed that all fractions of the mixture decoction significantly inhibited the expression of TNF-α, IFN-γ, IL-1β and IL-4 in U937 cells in a concentration of 25µg/mL.

Inhibition of COX-2 gene expression in LPS stimulated THP-1 macrophages by *Epipremnum pinnatum*

San-Po Pan, Rudolf Bauer

Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz

Cyclooxygenase -2 (COX-2) an important source for prostaglandin (PG) formation is responsible for many pathologic conditions and is often highly expressed in many inflammatory and proliferative diseases. Common drugs on the market targeting COX-2 e.g., ASS, Ibuprofen, Dexamethasone are often prescribed but prolonged administration can cause adverse effects such as gastric lesions, bleedings and nephrotoxicity. Therefore, identification of novel lead compounds from natural sources targeting COX-2 is needed [1-2].

Epipremnum pinnatum (Araceae) is traditionally used as an analgesic, anti-inflammatory, anti-cancer drug in various areas of Asia [3]. With a PMA differentiated, LPS stimulated THP-1 inflammation model [4], we evaluated the influence of leaf extracts (n-hexan, dichlormethan, methanol) on COX-2 gene expression. At the concentration of 20µg/ml, methanol extract showed potent COX-2 mRNA inhibition (54.3% +/- 9.2) leading to further fractionation with Silica gel 60 and Sephadex LH 20 gaining 3 active fractions (S2 = 71.8 +/- 2,6, S3 = 81.7 +/- 3.5, S4 = 63.3 +/- 1.8).

Taken together, we could demonstrate that the methanol extract of *Epipremnum pinnatum* exerts strong *in vitro* COX-2 gene expression inhibition. Our further aim is to identify and isolate compounds responsible for the activity.

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