Phospholipid Research Center news

First Announcement!
The Phospholipid Research Center organises its first symposium on “Phospholipids in Pharmaceutical Research” on May 10-11, 2009 in Heidelberg.

Aspects

Phospholipids are already widely used in registered pharmaceutical formulations worldwide:

Lipid emulsions with phospholipids are used for parenteral nutrition and as drug delivery systems for lipophilic actives as for example propofol. The side effects of drugs can be considerably reduced by being packaged into liposomes, consisting of phospholipids. Examples are amphotericin B and doxorubicin.

However, their use could still be further extended as they might be able to replace almost all other emulsifiers and surfactants. Phospholipids are degraded by the body, can be well tolerated, are toxicologically harmless and can be used in suitable compositions in all systems.

But phospholipids can also act as active agents in pharmaceutics. In addition to the treatment of fatty livers, the positive effect of phospholipids has already been detected regarding the lipid metabolism, damages of the skin and the gastrointestinal tract.

The symposium “Phospholipids in Pharmaceutical Research” picks up the development of new applications and allows for fundamental research of phospholipids as well.

Aim of the symposium is to provide a platform for discussion and contact throughout the spectra of interests of phospholipid scientists.

Preliminary Program

Sunday, May 10, 2009
Welcome Dinner in Heidelberg

Monday, May 11, 2009
In the morning
Oral presentations of invited speakers
- Dr. Francis J. Martin (formerly Alza Corp. and Liposome Technology Inc.)
- Prof. Dr. Christel Müller-Goymann (Technical University Braunschweig)
- and many more

In the afternoon
Plenary session with submitted oral presentations
- Current research projects
- Projects supported by the PRC

During coffee breaks
Poster presentations in the foyer

Phospholipid scientists are encouraged to submit their abstracts for oral or poster presentation.

Abstract submission and registration
www.phospholipids.net
Deadline: April 15, 2009

Meeting of the Scientific Board, July 7, 2008 in Braunschweig

Participants:
Prof. Alfred Blume (Scientific Board)
Prof. Heike Bunjes (University of Braunschweig)
Prof. Gert Fricker (Scientific Board)
Dr. Frank Martin (Scientific Board)
Prof. Christel Müller-Goymann (Scientific Board)
Dr. Ralf-Olaf Quinkert (Scientific Board)
Dr. Herbert Rebmann (PRC)
Dr. Katharina Sauter (PRC)
Mr. Armin Wendel (PRC)
Dr. Jürgen Zirkel (PRC)
At the last meeting of the Scientific Board in Braunschweig, it was decided to support the following submitted research proposals:

- Sandra Herrmann, LMU München: “Vesicular phospholipid gels as new delivery platform for pharmaceutical proteins”
- Rolf Schubert, University of Freiburg: “Caco-2 cell studies on lipid-mediated intestinal adsorption of active substances”

To simplify the applications in future, a general form was advised. The proposal (4-5 pages in length) should be divided into the following parts:

1. Abstract
2. Introduction to the topic
3. Aim of the project
4. Methods
5. Work plan
6. Timeline
7. Costs

An annual report should reveal the progress of the funded project.

For more information about the funding of projects, please visit our website: www.phospholipids.net.

### Industry news

#### PLx Pharma: Bioequivalence of Aspirin and Aspirin-PC

PLx Pharma Inc. announced that it has successfully completed a clinical trial of PL 2100, also known as Aspirin-PC and demonstrated its bioequivalence with regular aspirin. This trial demonstrates PL 2100 Aspirin-PC may bridge to the safety and efficacy of aspirin for prescription (Rx) treatment and prevention of secondary prevention of stroke and myocardial infarction and over-the-counter (OTC) analgesic and fever indications.

#### Transave: Positive phase II results for once-daily Arikace(TM)

Transave Inc. reported positive results from a Phase II clinical trial on its lead investigational drug, Arikace(TM) (liposomal amikacin for inhalation). The compound is being developed for the treatment of cystic fibrosis (CF) patients who have lung infections due to the bacterium Pseudomonas aeruginosa. The Phase II data indicated that Arikace, delivered once daily for 28 consecutive days, produced a significant improvement in lung function, was well-tolerated, and had a side-effect profile comparable to placebo. The Phase II trial was a randomized, double-blind, placebo-controlled study of 64 patients from 15 centers in Europe.

#### Doxorubicin liposome injection (Doxil) approved for AIDS-related Kaposi’s sarcoma

The FDA approved a new indication for doxorubicin HCl liposome injection (Doxil; Alza Corp), allowing its use for the treatment of AIDS-related Kaposi’s sarcoma after failure of previous systemic chemotherapy or intolerance to such therapy.

#### Alocrest: Liposomal injection with Optisome

Phase 1 data for Alocrest demonstrated prolonged stable disease and a solid tolerability profile at an MTD comparable to unencapsulated vinorelbine in heavily pre-treated patients. Alocrest is a novel sphingomyelin/cholesterol liposome-encapsulated vinorelbine tartrate formulation. Vinorelbine, a semi-synthetic vinca alkaloid, is a microtubule inhibitor that has been approved for use as a single agent or in combination with cisplatin for the first-line treatment of advanced non-small cell lung cancer. In several countries outside the United States, vinorelbine is also approved for the treatment of advanced stage breast cancer. Preclinical comparison data between commercially available vinorelbine tartrate injection (unencapsulated) and...
Alocrest demonstrated that Alocrest has improved pharmacokinetic properties, including an approximately 10-fold increase in preferential accumulation in tumors, and an improved therapeutic index.

Literature report

Nonviral nanoscale-based delivery of antisense oligonucleotides targeted to hypoxia-inducible factor 1 alpha enhances the efficacy of chemotherapy in drug-resistant tumor


A multifunctional drug delivery system consisting of a lipid-based nanoparticle, a standard anticancer drug, and a short nucleic acid designed to augment the activity of that drug has demonstrated the ability to treat drug-resistant tumor cells. The work suggests a novel approach to treating drug-resistant tumors, the leading cause of cancer deaths. A signaling pathway was targeted that causes tumors to trigger new blood vessel growth and to develop resistance to many anticancer drugs. To shut down this pathway, an antisense oligonucleotide was developed that would bind to and inactivate messenger RNA coding for a protein known as hypoxia-inducible factor-1α, which, when present, activates the targeted pathway.

DNA that is dispersed in the liquid crystalline phases of phospholipids is actively transcribed


A 4.3 kbp linearised T7 DNA plasmid is actively transcribed when it is dispersed in the hexagonal liquid crystalline phase of dioleoylphosphoethanolamine (DOPE).

Reversible, reagentless solubility changes in phosphatidylcholine-stabilized gold nanoparticles


Phosphatidylcholine (PC) is a versatile ligand for synthesizing gold nanoparticles that are soluble in either organic or aqueous media. A novel route is reported to organic-soluble, PC-stabilized gold nanoparticles that can be re-suspended in water after removal of the organic solvent. Similarly, it is shown that PC-stabilized gold nanoparticles synthesized in water can be re-suspended in organic solvents after complete removal of water. Without complete removal of the solvent, the nanoparticles retain their original solubility and do not phase transfer. This change in solvent preference from organic to aqueous and vice versa without the use of an additional phase transfer reagent is novel, visually striking, and of utility for synthetic modification of nanoparticles.

Human tumor nanoparticles induce apoptosis of pancreatic cancer cells


Using nanoparticles made from pieces of tumor cells, a new type of anticancer agent was developed that appears to stop tumor cell growth and proliferation. The investigators began their study by harvesting pieces of tumor cell membrane that bud off from pancreatic cancer cells. These exosomes resemble lipid-based nanoparticles known as liposomes, but the exosomes are loaded with various tumor cell membrane proteins. After purifying the nanoparticles, the researchers administered them to tumor cells, triggering cell death at a level proportional to the amount of nanoparticles.
added to the cells. The nanoparticles had no effect when added to normal cells.

**Rapid distribution of liposomal short-chain ceramide in vitro and in vivo**
Ceramide, an endogenous sphingolipid, has demonstrated antineoplastic activity in vitro and in vivo. However, the chemotherapeutic utility of ceramide is limited, due to its insolubility. To increase the solubility of ceramide, liposomal delivery systems have been utilized. The objective of the present study was to characterize the pharmacokinetics and tissue distribution of C6-ceramide and control (non-C6-ceramide) nanoliposomes in rats, using [(14)C]-C6-ceramide and [(3)H]-DSPC (distearoyl-phosphatidylcholine) as tracers of the ceramide and liposome components, respectively.

**Oral Phosphatidylcholine preserves the GI mucosal barrier during LPS-induced inflammation**
The hydrophobic surface layer of the gastrointestinal (GI) tract, which has been attributed to the presence of phosphatidylcholine (PC) in the mucus gel, protects the mucosa of the GI tract and is disrupted by parenteral LPS treatment. The potential for repletion of this layer as a means to prevent LPS-induced GI injury was investigated. Rats were treated orally with PC 1 h before LPS (i.p.). Gastric and ileal tissues were assessed for changes in permeability 5 h later, and gastric fluid was analyzed for signs of GI-related LPS effects (bile acid reflux, increased volume, and pH) and gastric injury (bleeding).

**Clinical Trial: comparison of Ibuprofen-PC and ibuprofen on the GI safety and analgesic efficacy in osteoarthritic patients**
Results: Ibuprofen-PC and ibuprofen provided similar bioavailability/therapeutic efficacy. In the evaluable subjects a trend for improved GI safety in the Ibuprofen-PC group compared with ibuprofen was observed, that did not reach statistical significance. However, in patients >55 years of age, a statistically significant advantage for Ibuprofen-PC treatment vs. ibuprofen in the prevention of NSAID-induced gut injury was observed with increases in both mean Lanza scores and the risk of developing > 2 erosions or an ulcer. Ibuprofen-PC was well tolerated with no major adverse events observed. Conclusions: Ibuprofen-PC is an effective osteoarthritic agent with an improved GI safety profile compared to ibuprofen in older OA patients, who are most susceptible to NSAID-induced gastroduodenal injury.

**A convenient method for lecithin purification from fresh eggs**
The increasing demand for fatty acid-free lecithin required modifications in existing purification methods. In this technical note we describe a purification procedure with the following steps: a) homogenization and extraction of yolks obtained from fresh eggs with acetone, b) solubilization with ethanol and solvent elimination and c) repeated solubilization/precipitation with petroleum ether/acetone. This crude extract was chromatographed on neutral alumina, which was exhaustively washed with chloroform before elution with chloroform:methanol, allowing the sequential sepa-
ration of fatty acids and lecithin. Chromatographic behavior and mass spectra of the product are presented. This fast procedure yields fatty acid-free lecithin at a competitive cost.

Update on vegetable lecithin and phospholipid technologies


This paper reviews the production technologies for sourcing lecithins from the oil-bearing seeds soybean, rapeseed and sunflower kernel. The phospholipid composition is measured by newly developed HPLC-LSD and 31P-NMR methods. The phospholipid compositions of the three types of lecithin show small differences, while the fatty acid composition is largely equivalent to the oil source. Regulatory specifications (FAO/WHO, EU, FCC) and DGF and AOCS analytical methods for product quality are compiled. Phospholipid modifications by enzymatic hydrolysis, solvent fractionation, acetylating and hydroxylation processes result in lecithins with specific enhanced hydrophilicity and oil-in-water emulsifying properties.

Lysosphospholipid metabolism facilitates Toll-like receptor 4 membrane translocation to regulate the inflammatory response


In this article, it is shown that lysophosphatidylcholine acyltransferase (LPCAT) regulates inflammatory responses to LPS and other microbial stimuli. Specific inhibition of LPCAT down-regulated inflammatory cytokine production in monocytes and epithelial cells by preventing translocation of TLR4 into membrane lipid raft domains. The observations demonstrate a new regulatory mechanism that facilitates the innate immune responses to microbial molecular patterns and provide a basis for the anti-inflammatory activity observed in many phospholipid metabolites. This provides the possibility of the development of new classes of anti-inflammatory and antisepsis agents.

The oral absorption of phospholipid prodrugs: in vivo and in vitro mechanistic investigation of trafficking of a lecithin-valproic acid conjugate following oral administration.


The mechanisms involved with the trafficking of this conjugate following oral administration in the gastrointestinal (GI) lumen, within the enterocyte and further were investigated. A phospholipid-valproic acid conjugate (DP-VPA) was utilized as a model molecule. Direct conjugation between the drug and the phospholipid produces a complex having unique absorption properties that include: (1) a stable complex that does not undergo degradation in the GI tract; (2) permeation through the gut wall and entering intact to the enterocyte; and (3) association with chylomicron in the enterocyte and reaching the systemic circulation via the lymphatic route. These unique properties may be of interest in drug delivery.

Advances in planar lipid bilayers and liposomes: Vol. 4


The lipid bilayer is central to life, as all living organisms possess a lipid bilayer structure, thereby underlying the lipid bilayer principle of biomembranes. The lipid bilayer principle and its applications are the main theme of this new book series. This new series on bilayer lipid membranes (BLMs and liposomes) include invited chapters on a broad
range of topics, from theoretical investigations, specific studies, experimental methods, to practical applications. Written for newcomers, experienced scientists, and those who are not familiar with these specific research areas, the Series covers all aspects of lipid bilayer investigations, both fundamental and applied. It covers a broad range of topics ranging from theoretical research, specific studies, experimental methods, to practical applications. It contains authoritative timely reviews by experts in this field. It is an indispensable source of information for new scientists.

Impact of Essentiale L on ethanol-induced changes in rat brain and erythrocytes


The effect of Essentiale L, a mixture of polyenylphospholipids from soybeans, was investigated on oxidative stress in various brain regions, on erythrocytes (RBC) and on RBC membrane composition in ethanol-administered rats. The findings suggest that Essentiale L, a therapeutic adjunct for liver diseases, also has bioprotective effects on non-hepatic tissues and cells.

Binding of nonsteroidal anti-inflammatory drugs to DPPC: structure and thermodynamic aspects


The effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on the phase transition and phase properties of 1,2-dipalmitoylphosphatidylcholine (DPPC) has been investigated in both 2D and 3D model systems. The results give indications of the role of the membrane/NSAID interactions that might also be important for NSAID gastric injury.
includes rabbit brain. The synthetic phospholipids of the mixture include palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylserine (POPS), and a phosphatidylalcohol. The phosphatidyl alcohol includes dioleoylphosphatidylethanol, dioleoylphosphatidylethanol, dioleoylphosphatidylethanol, dioleoylphosphatidylethanol, and dioleoylphosphatidylethanol.

**US Pat. Appl. 20080187583; August 7, 2008**

Tablet containing hydrogenated phospholipids

Recently, it was found that hydrogenated phospholipids, perorally administered, can be therapeutically effective with respect to cancer disease and its subsequent effects. The invention provides a process for preparing a tablet containing hydrogenated phospholipids and the tablet obtainable by such process.

**Compilation of literature: Torsten Kromp**

**Conference report**

The **2nd Midnight Sun Meeting on Drug Transport & Delivery in Tromsø, Norway, June 25-27, 2008**

On June 25-27, 2008, the 2nd Midnight Sun Meeting on Drug Transport & Delivery took place at the Institute of Pharmacy of the University of Tromsø, Norway. The Drug Transport & Delivery group at Tromsø University was celebrating its 10th anniversary in 2008. Co-organiser was the Nordic Chapter of the Controlled Release Society, arranging their annual meeting 2008 in conjunction with this conference. The aim of the meeting was to provide an informal forum for scientists from both academia and industry working on drug transport research as well as on drug delivery. In 2008, about 90 scientists, the majority of them from Scandinavia and Germany, participated in the meeting.

The scientific program was opened by Annette Bauer-Brandl, one of the organisers. Each session about solid state, drug transport and drug delivery, respectively, consisted of one or two invited lectures followed by submitted oral presentations. The poster session took place before the welcome reception at the first evening of the meeting. Both poster and oral presentations spanned a wide field of pharmaceutical topics ranging from tablet properties to clinical results and stimulated lively discussions.

The Phospholipid Research Center presented two posters of funded projects. One poster was together with Prof. Fricker, Heidelberg, about the "Oral bioavailability of pharmaceutical actives by NanoSolve". Another poster was from Dr. Pütz, Freiburg, about his project "Phosphatidylcholines in anticancer drug delivery: mere innocent bystanders?" Both posters can be downloaded at the website www.phospholipids.net.

From the submitted presentations, Christopher Bachran’s (Freie Universität Berlin) talk “Combined Application of saponins and chimeric toxins for tumor treatment in mice” and the poster “Controlled release of drugs from liposomes by low-frequency ultrasound: Effect of PEGylation” of Avi Schröder (Hebrew and Ben Gurion University of Massouot Yithak, Israel) were selected as the best oral and poster presentation, respectively.

The conference dinner took place in the restaurant Arctandria downtown at the harbour. Regional delicacies like whale, seal and reindeer were served.

The participants perceived the meeting as informative in a relaxed atmosphere. Unfortunately the midnight sun did not show its face during the meeting. Only those people who stayed over the weekend were rewarded with a beautiful sight.
PEG-Phospholipids

Since 1990 a new type of phospholipids for the preparation of long circulating liposomes has been synthesized by coupling polyethyleneglycolmonomethylether (mPEG) as second polar head to phosphatidylethanolamine (PE). The most important phospholipid conjugates are mPEG 2000-DSPE and mPEG 5000-DPPE prepared from mPEG of an average molecular weight of about 2000 or 5000, respectively. The molecular structure of mPEG 2000-DSPE as sodium salt is depicted in Fig.1.

PEG-phospholipids prepared from phospholipids with saturated fatty acid chain as DSPE or DPPE are soluble in water, acetone and ethanol as well.

The introduction of PEGylated phospholipids into phospholipids bilayers prepared from phosphatidylcholine (e.g. DSPC or hydrogenated soya phosphatidylcholine) and cholesterol results in sterically stabilized structures which enhance the blood circulating time of liposomes considerably. The so-called stealth effect may be explained by the presence of the large hydrophilic head covalently bound to the phospholipids which changes the interaction of the liposomes with blood and protects them from undesired uptake by the macrophages.

*In vitro* studies using liposomes with PEGylated phospholipids have shown increased stability e.g. in tests as plasma induced leakage, agglutination, Ca$^{2+}$ induced fusion, enzyme degradation and others. PEG-phospholipid containing liposomes have shown very similar safety properties as conventional liposomes in *in vivo* studies.

The prolonged blood circulation time, the different bio-distribution and pharmacokinetics are the main advantages for pharmaceutical application of “stealth” liposomes in drug delivery systems to improve the circulation of the encapsulated drug. PEG-phospholipids are further of interest for drug targeting as various ligands (antibody, protein, peptide or carbohydrate) may be coupled to the polymeric side of the liposomes.

*Fig. 1:* 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000], sodium salt (mPEG 2000-DSPE).
## Analytical column

### Viscosity

For liquid lecithins and phospholipids the viscosity is important for the handling and transport.

Liquid lecithins with less than 10,000 Pa·s can easily be pumped and transported in tankcars. Lecithins with higher viscosity have to be warmed carefully to avoid hydrolysis and burning.

There are different methods for the testing of the viscosity of lecithins. The best methods for the measurement of the viscosity of lecithins are working with rotations viscosimeters according to DGF F-I 2a (00) or DIN 53018/019 or ISO 6388.

The viscosity of lecithins is influenced by the content of phospholipids. All products with a higher content than 65 % of these products are more or less highly viscous or solid. But the viscosity can be reduced by addition of oil or better free fatty acids or by alcohols like propandiol or ethanol and increases with the content of water.

Pure de-oiled natural phospholipids are solid.

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