Newsletter

Volume 9, Number 1

December 2015

Phospholipid
Forschungszentrum/Research Center
Heidelberg

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Introduction

In 2015 the Phospholipid Research Center organized its 4th International Symposium on Phospholipids in Pharmaceutical Research on September 21st and 22nd, 2015 in Heidelberg with overwhelming success.

During the Symposium for the first time the Thudichum Award was donated to support young talents in pharmaceutical phospholipid research. The details on the ceremony and award winners you will find below.

Two Scientific Advisory Board meetings took place in 2015. You will find the major decisions of these meetings in this Newsletter.

The General Assembly of the Phospholipid Research Center took place, immediately after the Symposium on September 22, 2015. The details on the results of the General assembly you will find below as well.

It is important to note for the phospholipid research community that Dr. Paola Luciani, member of our association, has been appointed October 2015 as endowed professor (supported by Lipoid GmbH) for "Phospholipids in Drug Development" at the Friedrich Schiller University of Jena, Germany. We would like to congratulate her to this appointment and wish her all the best with her research endeavours.

Finally, we thank all members for their valuable contributions of 2015 and wish all of you a Happy and Successful 2016!

Peter van Hoogevest

Phospholipid Research Center News

Meetings of the Scientific Advisory Board

The Meetings of the Scientific Advisory Board took place on January 19th, 2015 (Phospholipid GmbH, Köln) and on June 29th, 2015 (University Heidelberg, hosted by Prof. Fricker).

Participants:

Prof. Dr. Alfred Blume (Scientific Board)
Prof. Dr. Gert Fricker (Scientific Board)
Dr. Frank Martin (Scientific Board)
Prof. Dr. Christel Müller-Goymann (Scientific Board)
Dr. Ralf-Olaf Quinkert (Scientific Board)
PD Dr. Peter van Hoogevest (Managing Director, PRC)
Dr. Herbert Rebmann (President, PRC)
Mr. Armin Wendel (Vice President, PRC)
Dr. Jürgen Zirkel (PRC)

The Phospholipid Research Center received in total 19 proposals for projects to be funded in 2015. The Scientific Board approved 6 proposals.

The next meeting will take place on January 18th, 2016 at Lipoid GmbH in Ludwigshafen am Rhein. Proposals should be sent before December 31st, 2015.

For more information about the funding of projects, and how to submit a research proposal, please visit our website: www.phospholipid-institute.com .

Ongoing funded projects

The following 22 projects are at present ongoing:

"Phospholipid Based Solid Dispersions for Enhanced Oral Absorption of Poorly Soluble Drugs".
Prof. Annette Bauer-Brandl, University of Southern Denmark, Odense, Denmark.

"Improving treatment of pediatric sarcoma through targeted liposomal drug delivery".
Dr. Michele Bernasconi, University Childrens Hospital Zurich, Switzerland.

"Phospholipids as functional excipient in solid oral dosage forms".
Prof. Roland Bodmeier/Dr. Martin Koerber, FU Berlin, Germany.

"Phospholipid liquid fill formulations for hard gelatin capsules".
Prof. Heike Bunjes, University Braunschweig, Germany.

"Investigation of cochleate formulations and cochleate-cell membrane interactions".
Prof. Alfred Fahr, University of Jena, Germany.

„The investigation of the interaction of liposomal formulations containing poorly water soluble drugs with human plasma".
Prof. Alfred Fahr, University of Jena, Germany

"Investigations on the retention of lipophilic drug compounds within liposomal drug carriers".
Dr. Stephan Hupfeld, University Oslo, Norway, Prof. Martin Brandl, University of Southern Denmark, Odense, Denmark.

"Phospholipid-Functionalized Calcium Carbonate Based DD System to Improve the Bioavailability of PWSD"
Prof. Huwyler, Dr. Gabriela Québatte, University Basel, Switzerland.

"Interactions of the tumor-targeting vector peptide pHLIP with phospholipids".
Prof. Sandro Keller, University Kaiserslautern, Germany.

"Inhibition of Akt by polyunsaturated phosphatidylcholine – a novel approach for anti-cancer therapy.
Dr. Andreas Koeberle, University Jena, Germany.

"Study of mono-acyl lecithin complexes of poorly water soluble compounds; which compounds benefit particularly from dissolution enhancement regarding improved BAV?"
Prof. Martin Kuentz, University of Applied Sciences and Arts Northwestern Switzerland, Muttenz, Switzerland.

"Interaction of phospholipids with the skin in human subjects",
Dr. Majella Lane, University London, United Kingdom.

"In vivo detoxification of alcohol using phospholipid supported bioreactors".
Prof. Jean-Christoph Leroux, ETH Zurich, Switzerland.

"Phosphatidylserine enriched phospholipids as anti - inflammatory agents",
Prof. Karsten Mäder, PD Dr. Annette Meister, Univ. Halle-Wittenberg, Germany.

"Assessment and better prediction of acute hypersensitivity and complement involvement upon administering liposomal drug products to human subjects".
Dr. Bart. Metselaar, University Twente, The Netherlands, Prof. Janos Szebeni, Semmelweis University, Budapest, Hungary.

"Elucidating the use of lyso-phospholipids in oral self-nano-emulsifying drug delivery systems".
Prof. Anette Müllertz, University Copenhagen, Denmark.

"Development of colloidal carrier systems (micro-emulsions) on the basis of phospholipids for dermal application".
Profs. Reinhard Neubert/Johannes Wohlrab and Prof. Gerald Brezesinsky, University Halle/Potsdam, Germany.

"Design of well-defined liposomes to target tumor associated M2 macrophages".
Dr. Jai Prakash, University Twente, The Netherlands.

"Phospholipid micro-emulsions for the dermal application of proteins: Prediction of in-vivo skin tolerability with full-thickness human skin equivalent".
PD Dr. Regina Scherließ, Kiel University, Germany.

"Impact of Phospholipid Oxidation on Biophysical Properties of Membranes".
Prof. Motomu Tanaka, University Heidelberg, Germany.

"Development of NLCs and nanoemulsions with monoacyl-phospholipids and investigation of their skin Interaction".
Prof. Claudia Valenta, University Vienna, Austria.

The project on "Treatment and diagnosis of early gastric cancer with lipid based formulated hypericin by applying photodynamic therapy", Dr. Frieder Helm, Prof. Gert Fricker, University Heidelberg, Germany, was approved, but the contract is pending.

Characteristics of Projects
Country origin
As derived from the presently 22 approved and ongoing projects, the universities that are being funded are located in: Germany (11), Switzerland (4), Denmark (3), The Netherlands (2), UK (1) and Austria (1).

Research fields
The projects were related to the oral (8), parenteral (8) or topical (skin) (4) administration and analytical aspects (2)

Workshop proposal

The proposal made by Prof. Keller, University Kaiserslautern for funding of the Meeting of the Membrane Sections of the French and German Biophysical Societies "Biophysics of Protein–Membrane Interactions: From Model Systems to Cells" on 11-14, April 2016 in Bad Herrenalb, Germany was approved for 2000 €.

Publications
Following publications, related to projects supported by the Phospholipid Research Center were made during 2015:

Gautschi N, van Hoogevest P, Kuentz M 2015. Amorphous drug dispersions with mono- and diacyl lecithin: On molecular categorization of their feasibility and UV dissolution imaging. Int J Pharm June 27. pii: S0378-5173(15)30006-5.

Hinna A, Steiniger F, Hupfeld S, Stein P, Kuntsche J, Brandl M 2015. Filter-extruded liposomes revisited: a study into size distributions and morphologies in relation to lipid-composition and process parameters. J Liposome Res, Early Online: 1–10 DOI: 10.3109/08982104.2015.1022556.

Holzschuh S, Kaess K, Fahr A, Decker C 2015. Quantitative In Vitro Assessment of Liposome Stability and Drug Transfer Employing Asymmetrical Flow Field-Flow Fractionation (AF4). Pharm Res Nov. 2015 DOI: 10.1007/s11095-015-1831-y.

Hoppel M, Juric S, Ettl H, Valenta C 2015. Effect of monoacyl phosphatidylcholine content on the formation of microemulsions and the dermal delivery of flufenamic acid. Int J Pharm 479(1):70-6.

Klingler J, Vargas C, Fiedler S, Keller S 2015. Preparation of ready-to-use small unilamellar phospholipid vesicles by ultrasonication with a beaker resonator. Anal Biochem 15:477:10-2.

Korytowski A, Abuillan W, Makky A, Konovalov O, Tanaka M 2015. Impact of Lipid Oxidization on Vertical Structures and Electrostatics of Phospholipid Monolayers Revealed by Combination of Specular X-ray Reflectivity and Grazing-Incidence X-ray Fluorescence. J Phys Chem DOI: 021/acs.jpcb.5b04451

Oral Presentations: Several researchers gave oral presentations during the Symposium on their recent finding of their projects funded by the Phospholipid Research Center.

Posters: Also many posters related to funded research projects were presented at the Symposium.

Symposium 2015

The Phospholipid Research Center Heidelberg, organized its "4th Symposium on Phospholipids in Pharmaceutical Research" from 21st to 22nd September 2015 at facilities of the University of Heidelberg, Germany. 170 researchers from all over the world attended the meeting. Eighteen seminars given by international experts in the field and 67 posters were presented.

The symposium was devoted to latest advances of the parenteral, oral and topical (skin and the lung) administration of dosage forms with phospholipids. The symposium was concluded with a Young Session chaired by Prof. Müller-Goymann at which six promising young scientists got the opportunity to present their posters, which were, because of their quality, preselected by the Scientific Advisory Board.

The meeting was very successful. We had good discussions and a pleasant social program with an excellent dinner at the famous Castle of Heidelberg.

Poster Award 2015

The following three researchers were selected by the Scientific Advisory Board for the Poster Award for best posters and poster presentations:

Mr. Johannes Klingler, Technical University Kaiserslautern, Germany, "Morphology control paves the way for drug-delivery applications of phospholipid bicelles".

Dr. Klazina Kooiman, Erasmus MC, Rotterdam, The Netherlands, "Fluorescence high-speed microscopy reveals dynamic behavior of lipid coating on ultrasound contrast agents in an ultrasound field".

Ms. Agatha Korytowski, University Heidelberg, Germany, "Impact of lipid oxidization on physical properties of phospholipid monolayers and membranes".

The Poster Award winners (from left to right): Mr. Johannes Klingler, Dr. Klazina Kooiman, Ms. Agatha Korytowski.

Thudichum Award 2015

Johann Ludwig Wilhelm Thudichum, also known as John Louis William Thudichum (August 27, 1829, Büdingen – September 7, 1901, London) was a German-born physician and biochemist. From 1847 he studied medicine at the University of Giessen, where he worked in the laboratory of Justus von Liebig (1803–1873). In 1853 he moved to London, where he worked for the remainder of his career.
Thudichum was a pioneer of biochemistry, and a founder of "brain chemistry". He is credited with conducting chemical analyses of over one thousand human and animal brains. In his research, he was the first to isolate and characterize numerous compounds including phospholipids and related compounds of the brain. In 1884 he explained his findings in a publication titled "A Treatise on the Chemical Constitution of the Brain", a book that was widely criticized and rejected at the time by many in the scientific community. After his death, Thudichum's discoveries were realized to be important scientific contributions to the study of the chemical and molecular composition of the brain and other organs. To honor that Prof. Thudichum was the first one who recognized the physiological importance of phospholipids, the award donated by the Phospholipid Research Center is named “Thudichum Award”.

During the Symposium for the first time the Thudichum Award was donated to support young talents in the pharmaceutical phospholipid research field in appreciation of outstanding related publication(s) in pharmacy with emphasis on recent significant contributions brought about by highly innovative research to understand and to effectively apply phospholipid excipients in the research and development of dosage forms for any route of administration.

The members of the committee which assessed the applications for the Award and selected the Award winner were Prof. Chezy Barenholz (Hebrew University, Jerusalem, Israel) and Prof. Daan Crommelin (Utrecht University, The Netherlands), both well known for their impressive contributions in the pharmaceutical phospholipid research field.

The Phospholipid Research Center granted the Thudichum award of 2015 to Dr. Margaret Nancy Holme of the Imperial College London, UK, because of her innovative work on shear-stress sensitive lenticular vesicles for targeted drug delivery. A relevant publication is: Holme MN, Fedotenko IA, Abegg D, Althaus J, Babel L, Favarger F, Reiter R, Tanasescu R, Zaffalon PL, Ziegler A, Müller B, Saxer T, Zumbuehl A 2012, Shear-stress sensitive lenticular vesicles for targeted drug delivery, Nature Nanotech, 7, 536-543.
Prof. Crommelin gave the laudation and congratulated Dr. Margaret Holme to the winning of the first Thudichum Award.

Prof. Daan Crommelin handing over the Thudichum Award to Dr. Margaret Holme

General Assembly

This time some important decisions were made by the General Assembly.

The By-laws of the association were optimized and elaborated.

Dr. Herbert Rebmann and Mr. Armin Wendel resigned at their own request from the Board. The General Assembly acknowledged their many valuable contributions to the Phospholipid Research Center.

Unanimously, the General Assembly elected Dr. Jürgen Zirkel as President and Prof. Dr. Alfred Blume as Vice-President for a term of office of three years.

To honour his valuable contributions and support of the Phospholipid Research Center, Dr. Herbert Rebmann was elected unanimously by the General Assembly as Honorary President.

The General Assembly further decided that individual projects will be funded partially or completely. The extent and duration of the financial support depends on expected research efforts and attractivity of the proposal. Normally the funding of a PhD Thesis project is maximally 2/3 of the country-specific costs per year, for a maximally three years period. Post-doc projects are completely funded for one year. Institute overhead costs are, however, not included in the funding.

Industry News

Merrimack announces U.S. FDA approval of ONIVYDE™ (irinotecan liposome injection) for the treatment of patients with metastatic pancreatic cancer. Press release, Oct. 22, 2015.

Merrimack today announced that ONIVYDE™ (irinotecan liposome injection) has been approved by the U.S. Food and Drug Administration (FDA) in combination with fluorouracil (5-FU) and leucovorin for the treatment of patients with metastatic adenocarcinoma of the pancreas after disease progression following gemcitabine-based therapy. "This is an important day for patients facing pancreatic cancer," said Andrea Wang-Gillam, M.D., Clinical Director of GI Oncology Program, at Washington University "With a long history of failed clinical studies in the post-gemcitabine setting, this approval is a significant achievement in the oncology community. It brings a new therapy to the many patients who are facing this aggressive disease and are in need of treatment options. The FDA approval is based on the results of an international Phase 3 randomized, controlled study (NAPOLI-1). In this study, ONIVYDE in combination with 5-FU and leucovorin achieved its primary

endpoint of a significant improvement in overall survival (p=0.014, unstratified HR=0.68, 95% CI: [0.50-0.93]) with a 45% improvement in median overall survival of 6.1 months for patients receiving the ONIVYDE combination regimen compared to 4.2 months for patients who received 5-FU and leucovorin alone.

The ONIVYDE combination also demonstrated improvement in progression free survival (3.1 months vs. 1.5 months, HR=0.55, 95% CI: [0.41-0.75]). The most common adverse reactions (> 20 %) of ONIVYDE were diarrhoea, fatigue/asthenia, vomiting, nausea, decreased appetite, stomatitis and pyrexia, and the most common severe laboratory abnormalities (> 10% Grade 3 or 4) were lymphopenia and neutropenia. There are approximately 49,000 patients diagnosed with pancreatic cancer each year in the United States, the overwhelming majority of whom have adenocarcinoma.

Celator® Pharmaceuticals announces positive recommendation from data and safety-monitoring board for Phase 3 study Of CPX-351 (VYXEOS™)- Company expects overall survival data in First Quarter of 2016.
Press release, Oct. 19, 2015.

Celator announced that the independent Data and Safety Monitoring Board (DSMB) for the Company's Phase 3 clinical study of CPX-351 (cytarabine:daunorubicin) Liposome for Injection (now referred to as VYXEOS™) has completed the finally planned safety review of all patients and has again recommended the study continue as planned without any modifications.

"Our confidence in the safety profile of VYXEOS is further strengthened with each positive DSMB recommendation," said Arthur Louie, Chief Medical Officer of Celator Pharmaceuticals. "The conduct of the Phase 3 study has gone remarkably well, having achieved each milestone on time or ahead of schedule. This final confirmation of safety for the Phase 3 study is a major step in the development of VYXEOS for patients with poor prognosis AML."

Mirna therapeutics announces the publication of new data supporting potential immune-related mechanism for anti-cancer activity of MRX34
Press release, November 23, 2015.

Mirna Therapeutics, Inc. (Nasdaq:MIRN), a clinical-stage biopharmaceutical company developing a broad pipeline of microRNA-based oncology therapies, today announced that the Journal of the National Cancer Institute (JNCI) published new preclinical data demonstrating a novel mechanism by which MRX34, the Company's lead product candidate, can stimulate the immune system to potentially induce an anti-tumor immune response. MRX34 is a double-stranded synthetic mimic of the naturally occurring tumor suppressor microRNA (miRNA) miR-34, encapsulated in the SMARTICLES® liposomal delivery formulation. miR-34 has been widely studied as a critical tumor suppressor microRNA and has been shown to be a key regulator of multiple oncogenes across multiple oncogenic pathways.

The new data indicate that miR-34 can also regulate anti-tumor immune functions by repressing PD-L1 (programmed death receptor ligand 1), an immune checkpoint signaling molecule that is upregulated by many tumor cells to escape the surveillance of the body's immune system. The researchers' conclusions suggest that the therapeutic potential of MRX34 derives from its ability not only to repress multiple oncogenes but also to block tumor evasion pathways.

The newly published study demonstrates that PD-L1 is negatively regulated by the tumor suppressor p53 via miR-34. In non-small cell lung cancer (NSCLC)

cells, mutated p53 was associated with lower expression levels of miR-34, consistent with previous data indicating that p53 directly induces the expression of the miR-34 gene. The authors also observed a concurrent increase of CD8+ tumor-infiltrating lymphocytes (TILs), and decrease of CD8+PD1- TILs, suggesting that MRX34 can alter immune cell profiles in the tumor and potentially reverse tumor immune evasion.

Literature alerts

Indocyanine green liposomes for diagnosis and therapeutic monitoring of cerebral malaria.
Portnoy E, Vakruk N, Bishara A, Shmuel M, Magdassi S, Golenser J, Eyal S 2016. Theranostics, 6(2): 167-176. doi: 10.7150/thno.13653.

Comments: the study demonstrates the diagnostic use of liposomes. Interestingly, in vivo imaging (possibly applicable in patients) and histological analyses are compared.

Cerebral malaria (CM) is a major cause of death of Plasmodium falciparum infection. Misdiagnosis of CM often leads to treatment delay and mortality. Conventional brain imaging technologies are rarely applicable in endemic areas. Here we address the unmet need for a simple, non-invasive imaging methodology for early diagnosis of CM. This study presents the diagnostic and therapeutic monitoring using liposomes containing the FDA-approved fluorescent dye indocyanine green (ICG) in a CM murine model. Increased emission intensity of liposomal ICG was demonstrated in comparison with free ICG. The Liposomal ICG's emission was greater in the brains of the infected mice compared to naïve mice and drug treated mice (where CM was prevented). Histological analyses suggest that the accumulation of liposomal ICG in the cerebral vasculature is due to extensive uptake mediated by activated phagocytes. Overall, liposomal ICG offers a valuable diagnostic tool and a biomarker for effectiveness of CM treatment, as well as other diseases that involve inflammation and blood vessel occlusion.

The safety of liposome bupivacaine following various routes of administration in animals.
Joshi GP, Patou G, Kharitonov V 2015. J Pain Res, 8:781-789.

Comments: This study demonstrates that the product Exparel, which has an attractive duration of action after post-surgical injection, is also well tolerated compared to the non-liposomal formulation of bupivacain.

This report presents results from four preclinical studies evaluating safety and pharmacokinetics (PKs) of liposome bupivacaine following intravascular (intravenous [IV], intra-arterial [IA]), epidural, and intrathecal administration in dogs.

Intravascular administration was initially tested in a pilot study to determine maximum tolerated doses, and then in an expanded study of systemic adverse effects and PKs. An epidural study compared properties of liposome bupivacaine alone and in combination with lidocaine/epinephrine vs bupivacaine HCl. Another study assessed effects after intrathecal administration.

In the initial intravascular studies, maximum doses at which no meaningful adverse events were observed with liposome bupivacaine were higher than for bupivacaine HCl (4.5 mg/kg IV vs 0.75 mg/kg IV, and 1.5 mg/kg IA vs 0.1 mg/kg IA, respectively). In the expanded intravascular study, there was no mortality or changes in pathology; adverse clinical signs included convulsions, lying on side, and decreased muscle tone (all were transient). In the epidural study, liposome bupivacaine was well tolerated at doses up to

the highest dose tested (40 mg), with no evidence of spinal cord damage and with less motor blockade than bupivacaine HCl 15 mg. Intrathecal administration of liposome bupivacaine 40 mg was not associated with meaningful safety concerns and resulted in less motor blockade than bupivacaine HCl 15 mg. PK analyses showed that maximum plasma bupivacaine levels following administration of liposome bupivacaine (4.5 mg/kg IV and 40 mg epidural) were similar to maximum plasma bupivacaine levels following a threefold lower dose of bupivacaine HCl (1.5 mg/kg IV and 15 mg epidural).

Liposome have a favorable safety profile compared with bupivacaine HCl when administered to dogs via intravascular, epidural, and intrathecal routes. This favorable safety profile is likely related to the liposome-bound nature of bupivacaine in the liposome bupivacaine formulation.

Liposomal Paclitaxel: Recent trends and future perspectives.
Sharma NK, Kumar V 2015
Int J Pharm Sci Rev Res, 31(1) 205-211.

Comments: Since many years liposomes are explored as carriers for paclitaxel. So far such a product has not been developed. It should be realized that replacing Cremophor by liposomes (phospholipids) as solubilizer, the anaphylactic side-effects of Cremophor can be eliminated.

One of the most promising anti-cancer agents paclitaxel, especially effective for treatment of ovarian and breast cancer, suffers from the problems like poor water solubility and low bioavailability. The current formulation available in a non-aqueous vehicle containing Cremophor EL® (polyethoxylated castor oil), on aqueous dilution, when given intravenously, may cause allergic reactions and precipitation. The lack of any appropriate delivery vehicle limits and delays the extensive clinical use of this drug. Hence, the development of alternate paclitaxel formulation with good aqueous solubility and lesser side effects is highly recommended. Researchers have so far explored different techniques including co-solvents, emulsions, micelles, liposomes, microspheres, nanoparticles, cyclodextrins, pastes, and implants. Among all these formulations, liposomes have been found to be highly effective against cancer. The current review encompasses the recent advancements in the delivery of paclitaxel by using liposomes. The focus is on searching future prospective for the safe and effective delivery of paclitaxel.

Subcutaneously injected ivermectin-loaded mixed micelles: formulation, pharmacokinetics and local irritation study.
Dong J, Song X, Lian X, Fu Y, Gong T 2015. Drug Deliv, 21:1-8.

Comments: This study demonstrates the usefulness of phospholipid containing mixed micelles for efficient solubilization of the lipophilic drug ivermectin.

Clinical application of ivermectin (IVM) is limited by several unfavorable properties, induced by its insolubility in water. Slight differences in formulation may change the plasma pharmacokinetics and efficacy. In this study, an IVM-loaded Soy phosphatidylcholine-sodium deoxycholate mixed micelles (IVM-SPC-SDC-MMs) were developed to improve its aqueous solubility, aiming to make it more applicable for clinical use. First, IVM-SPC-SDC-MMs were prepared using the co-precipitation method. After formulation optimization, the particle size was 9.46 ± 0.16 nm according to dynamic light scattering. The water solubility of IVM in SPC-SDC-MMs (4.79 ± 0.02 mg/mL) was improved by 1200-fold, comparing with free IVM (0.004 mg/mL). After subcutaneous administration, the pharmacokinetic study showed that IVM-SPC-SDC-MMs and commercially available IVM injection

were bioequivalent. Also, the local irritation study confirmed that IVM-SPC-SDC-MMs reduced side reactions of the commercially available IVM injection. These results indicated that IVM-SPC-SDC-MMs represented a promising new drug formulation suitable for subcutaneous delivery of IVM.

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