Program

11th European Congress of Toxicologic Pathology

A pathologist's view of animal models and *in-vitro* systems

Foto: Stad Gent – Dienst Toerisme

10th – 13th September 2013
Ghent, Belgium

organized under the auspices of the

European Society of Toxicologic Pathology

www.eurotoxpath.org

IATP Session (11th September 2013)
CNS Evaluation in Rodent Toxicity Studies

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Welcome

Dear colleagues and friends,

The European Society of Toxicologic Pathology (ESTP) is pleased to welcome you to the 11th EUROPEAN CONGRESS OF TOXICOLOGIC PATHOLOGY in Ghent, Belgium.

The congress Organizing Committees (Scientific and Local) have planned an excellent 4-day program on "A pathologist's view of animal models and *in-vitro* systems" and an additional IATP session will take place Wednesday afternoon (11th September) with focus on CNS evaluation in Rodent Toxicity Studies.

The sessions on "A pathologist's view of animal models and *in-vitro* systems" will include lectures addressing skin, CNS and liver models.

Tuesday, the 10th September 2013, we will focus on skin models dealing with irritation, psoriasis, wound healing, the relevance of dermal minipig studies and the correlation with the bovine cornea opacity and permeability test.

Wednesday morning, we will have a closer look at models of the Central Nervous system. This session will include an overview of MS pathogenesis, presentations on electrophysiology models, investigation of gene therapy and glial brain tumours. In the afternoon, the IATP session on CNS evaluation in Rodent Toxicity Studies will guide us through Brain Microanatomy and this session will provide an Overview of Non-Neoplastic Lesions of the Nervous System, the Use of the NTP Revised Rodent Brain Trimming Procedure for Routine Studies in CROs and Subsite Awareness in the NTP CNS Protocol.

The Liver will be the central topic for Thursday, September 12 filling our whole day from 2D and 3D in vitro models over gene expression, DILI, human liver pathology towards animal models (including e.g. metabolic syndrome, uPA+/+ SCID mice and zebrafish), biomarkers and PREDICT consortium results.

The closing session Friday, September 13, will focus on a SEND update, an INHAND update on GI tract (including salivary glands and exocrine pancreas) and hopefully lively interactive case presentations.

During Wednesday and Thursday, **posters** will be presented in the exhibition room.

We have organized some pleasant social events for you. On the evening of Tuesday 10th September we would like to invite you to join us at the **Welcome Reception**. It will take place in the Pacification hall of the Town Hall (see map for location). You will have the opportunity to meet colleagues and old friends, to chat and prepare yourself for the following days of the conference.

A boat trip through the historic heart of Ghent, will bring you to the Old Fish Market, where the **Conference dinner** will take place on Thursday the 12th of September. The monumental Old Fish market (1690) is located in the historic centre of Ghent next to the Gravensteen Castle, built of Tournai Limestone (1180). Please join us and use this informal evening to meet old and new friends and colleagues.

Please do not miss the **Annual General Assembly of the ESTP**. This will take place on Wednesday 12 September from 17.30h to 19.30h in the main session room. The agenda for this meeting is prepared by the secretary of the ESTP, Francesco Marchesi. A **Belgian beer tasting** is organized to follow the annual assembly meeting.

There will also be a **trade exhibition** related to the toxicopathology profession, where contract laboratory and other services, books, new equipment, etc. will be displayed in the halls of "De Handelsbeurs". Our exhibitors are a very important part of as well as a precious support for our meeting. Please take the time to visit their booths during the congress.

We look forward to meet you during the week and we wish you to enjoy both the congress and the lively city of Ghent.

On behalf of the Local and Scientific Organizing Committees

An Vynckier & Marjolein van Heerden

Table of Contents

Welcome 1

Table of Contents 2

General Information 6
- Congress Organizers 6
- Local Organizing Committee 6
- Scientific Organizing Committee 7
- Congress Venue 8
- Registration Desk 8
- Speaker Information 8
- Poster Presentation 8
- ESTP Interactive session 9
- Abstract Publication 9
- Awards 9
- Industry Exhibition 9
- Exhibition Quiz 9
- Additional Meetings 10
- Gastronomy 10
- Social Events 11
- Language 12
- Internet Access 12
- Messages 12
- Congress Bags 12
- Safety and Security 12
- Emergency Calls 13
- Currency 13
- Many thanks to our Exhibitors 14
- Many thanks to our Sponsors 15

Congress Program 16
- 10th September, Tuesday 16
- 11th September, Wednesday 17
- 12th September, Thursday 18
- 13th September, Friday 19

Table of Contents

Speaker Abstracts 20
S01: Models of diseased skin (including normal skin) 20
S02: Reconstructed human skin models: EU validated methods and predictive in vitro tools. 22
S03: Introduction to dermal studies and the minipig as a model in wound healing . . 23
S04: An *in vivo* evaluation model of cutaneous tolerance: development, use, limits and possible refinements 24
S05: Human skin equivalents: models for cutaneous biology, disease and drug screening 25
S06: The Bovine Cornea Opacity and Permeability test and the added value of histopathology 26
S07: Anti-angiogenic therapy of glial brain tumors; lessons learned from human glioma xenograft models. 28
S08: Potential and drawbacks of animal and in vitro models to study multiple sclerosis disease mechanisms 30
S09: Using electrophysiological approach to study the CNS white matter; in health and disease. 31
S10: Models of Insertional Mutagenesis following Gene Therapy – a Pathologist's View. 32
S11: In vitro and in vivo prediction of liver toxicity for early selection of drug candidates: Cellular Models & Molecular Tools 33
S12: Drug-induced acute liver damage – liver histopathology 34
S13: DILI, animal models and their use to establish biomarkers for human diagnostic purposes 35
S14: FP7 PREDICT-IV consortium – preliminary results from in vitro liver investigations 36
S15: Translational mechanism-based biomarkers for DILI in humans – from molecule to man 37
S16: Mouse models for cardiometabolic disease with specific focus on non-alcoholic steatohepatitis (NASH) 38
S17: uPA-SCID mice with a humanized liver: characterization and applications 39
S18: Predicting drug-induced hepatotoxicity in zebrafish larvae 40
S19: INHAND: Non-proliferative and proliferative lesions of the digestive tract 41
S20: Progress of INHAND and Collaboration with the FDA on SEND 43

Case Presentations 44
Case 1: Frederic Schorsch 44
Case 2: Vanessa Schumacher. 45
Case 3: Marjolein Van Heerden 47

Table of Contents

Case 4: Eva Tibaldi 49
Case 5: Tania Carvalho 51
Case 6: Monique Y. Wells 53
Case 7: Torrie A. Crabbs 55
Case 8: Alessandro Piaia 58
Case 9: Ursula Junker 60
Case 10: Franck Chanut 62

Poster Abstracts 64
P01: Non-invasive monitoring of Hepatic Stellate Cell activation and potential proliferation 64
P02: Induction of experimental autoimmune encephalomyelitis in cynomolgus monkey with recombinant human myelin oligodendrocyte glycoprotein in incomplete Freund's adjuvant 65
P03: Reproductive toxicology of soy milk in Swiss Albinos mice 66
P04: eTOX – Sharing preclinical data & building ontologies to enable better prediction of toxicity 67
P05: Occurrence of amphophilic-vacuolar renal tubule tumors and nephroblastomas in Sprague-Dawley Rats from sub-chronic toxicity studies 68
P06: Centriacinar lung lesions in control rats associated with oral gavage administration 69
P07: The effects of Protein Kinase C inhibitors on steroid hormones: a 2-week rat oral (gavage) investigative study focusing on pathology and hormone analysis in plasma and tissues 70
P08: Spontaneous complex odontoma arising from a molar tooth in a Syrian hamster 71
P09: Semi-automated quantitative image analysis for the glomerular markers desmin and WT1 in a Dahl salt sensitive rat model after unilateral nephrectomy – effect of Lisinopril and the combination with hydrochlorothiazide (HCTZ) on kidney function and morphology 72
P10: Acute oral, subchronic oral and dermal toxicity studies in Sprague-Dawley rats treated with agrochemical fungicide bellum 33.4 % 73
P11: Supporting the need for International Harmonization of safety assessment for Food Flavoring Substances 74
P12: Local effects of various microelectrodes implantation in rabbit muscles 75
P13: Possible contribution of cell proliferation to gene mutation following exposure to the hepatocarcinogen estragole 76

Table of Contents

P14: ZSF1 rats and kidney: Exacerbation of nephropathy by unilateral nephrectomy and efficacy of Lisinopril in ameliorating the lesions 77
P15: Strain differences in neurohistopathology and morphometry outcome in methylazoxymethanol treated rats 78
P16: Evaluating the eye irritation potential in BCOP assay by a semiquantitative histopathological method 79
P17: Modification of combined administration of CYP inducers in rat liver tumor promoting activity 80
P18: Nigella sativa oil protects against reproductive toxicity of acetamiprid insecticide in male rats 81
P19: Intraperitoneal administration of high doses of polyethylene glycol (PEG) causes hepatic subcapsular necrosis and low-grade peritonitis with a rise in hepatic biomarkers 82
P20: An overview of the modified National Toxicology Program approach to neuropathology 83
P21: Surface modification and different crystallinity of titanium dioxide nanoparticlescause similar histopathological lesions following inhalation in Wistar rats 84
P22: Detecting and quantifying liver pathologies using compact, high-resolution 3D MRI-based Histology 85
P23: The relevance of the guinea pig as species for repeated toxicity studies with human monoclonal antibodies 86
P24: Modifying effects of sulforaphane on diethylnitrosamine or furan induced liver carcinogenesis in rat 87
P25: Immunohistochemical characterization of spontaneous lymphomas in Sprague-Dawley rats: comparison with the human counterpart 88
P26: *In vivo* visualization of Pan-FGFR inhibitors mediated bone remodeling in rats by Osteosense®, a potential novel screening method 89
P27: The possible protective role of vitamins C and E in fluoride intoxicated rats 90
P28: Similar expression change of midline 1 on neuronal progenitor cells between developmental and adult-stage hypothyroidism in the hippocampal dentate gyrus of rats 91
P29: Intramuscularly injectable long-acting drug microsuspensions: Histopathological exploration of the local *in vivo* disposition and the implications for drug release 92
P30: FcRγIV and Clec4a3: novel markers of activated macrophages in mice 94
P31: Optical Coherence Tomography (OCT) and Fluorescein Angiography as a useful screening tool for ocular toxicity 95

General Information

Congress Organizers

Local Organizing Committee

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Janssen R&D

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General Information

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29646 Bispingen
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Fax: +49 5194 – 97 44 94
e-mail: pia.schroeder@solution-office.de

Congress Venue

HANDELSBEURS CONCERTZAAL
KOUTER 29
9000 GHENT
BELGIUM

The conference will take place in the Handelsbeurs in Ghent, located in the city center.

Registration Desk

The desk will be located at the ground floor in the foyer. All the congress documents can be picked up from the registration desk. An identification badge must be worn to enter all the congress sessions and events.

Registration is possible during the whole congress.

Opening hours of registration desk:
10th September 2013 11:00 h – 17:00 h
11th September 2013 08:00 h – 17:30 h
12th September 2013 08:00 h – 17:00 h
13th Sepetmber 2013 08:00 h – 11:00 h

Speaker Information

Video beamer and PC are available for presentations. Please turn in your presentations at the front desk before your session. Please use CD-ROM, USB stick or comparable format. The use of your own PC is not desired.

Poster Presentation

Posters will be exhibited during the entire Congress in the exhibitor area. Poster sessions are sheduled on Wednesday 10.30 h and Thursday 10.15 h (during the coffee breaks). It will also be possible to review the posters during coffee and lunch breaks.

Authors therefore are kindly requested to be at their posters during the poster session on Wednesday to answer potential questions.

The poster boards are kindly provided by

pds Intuitive Software Insightful Solutions

General Information

ESTP Interactive session

An interactive session on different cases of toxicologic pathology is organized on Friday.

Abstract Publication

Several presentations given at this congress will be published afterwards on the ESTP website in pdf-format.

Awards

- Novartis best publication Award
- SFPT Award for Best Poster
- IATP Charles Capen Trainee Award

The award ceremony is scheduled for Thursday 12th of September 15:15 – 15:35 h.
Please, participate.

Industry Exhibition

As in previous years, an exhibition featuring Pharmaceutical and Product Companies, Technical Equipment Companies and Medical Publishers will be held within the same setting as the conference. The entrance is free to those registered to the Conference and registered accompanying persons.

The exhibition will open on Wednesday, September 11, at 10:30 h and will then follow the same schedule as the conference. The exhibition will close after the afternoon coffee break of Thursday September 12.

The industry exhibition provides information about the newest technologies and developments available within our scientific area. The exhibiting companies have a unique possibility to efficiently reach their target customer. The ESTP values the support from exhibitors and believes that the on-site discussion and exchange of experience between exhibitors and the congress participants is of invaluable importance and benefit.

Please visit the booths of our exhibitors!

Exhibition Quiz

An exhibition quiz is organized. The documents needed for your participation will be handed out to you at the congress counter. The prize for the winner will be the book **„Toxicologic Pathology: Nonclinical Safety Assessment“**. The winner will be awarded on Thursday before the afternoon coffee break.

The prize is kindly sponsored by

EPL

General Information

Additional Meetings

ESTP Executive Committee board F2F meeting
Tuesday, September 10, 09:30 – 10:30 h in the room „Library“ in the conference venue.

ESTP Annual General Assembly
Wednesday, September 11, 17:30 – 19:30 h in the main conference room.

Frederic Schorsch: President of the ESTP

ESTP Scientific Organizing Committee Berlin 2014 F2F Meeting
Thursday, September 12, 07:00 – 08:30 h in the room „Library“ in the conference venue.

IFSTP F2F Meeting
Thursday, September 12, 12:30 – 13:30 h in the room „Library“ in the conference venue. Lunch will be available.

ESTP „possible presidents“ F2F Meeting
Friday, September 13, after the closing remark at 12:45 h in the room „Library“ in the conference venue.

Other additional Meetings
Other additional Meetings will be announced at the information board next to the registration desk in the meeting venue.

Gastronomy

Coffee, tea, refreshment beverages and pastries are served during the coffee breaks.
Lunch is provided during the lunch breaks on:

Wednesday, September 11
Thursday, September 12

General Information

Social Events

Welcome Reception (September 10th)

At the evening of Tuesday the 10th September we would like to invite you to join us at the Welcome Reception.

The reception will take place in the historic town hall of Ghent. The town hall is located in the city centre and within walking distance of the conference venue. The welcome reception will be in the „Pacificatiezaal".

Stadhuis
Botermarkt 1
9000 Gent

You will also have the opportunity to meet colleagues and old friends, to chat and prepare yourself for the following days of the conference. Join us!

Belgian Beer tasting (September 11th)

In the evening of the 11th September we would like to invite you to an informal get-together after the end of the conference in the exhibition area of the Handelsbeurs. We will offer some special beers from Belgium kindly provided by the local Brewery Huyghe, the Westmalle brewery and InBev Belgium nv.

Conference dinner (September 12th)

We welcome your attendance at the conference dinner on Thursday evening which will take place in the historical fish-market „Oude vismijn".

A taxi boat will depart from the landing point „Graslei" which is located in the centre of Ghent within walking distance of the conference venue (see Ghent map at the back of the book). The boat will depart at 19:30 h. From here we will make a short trip through the historical centre of Ghent and stop directly at the dinner venue.

Please join us at this beautiful evening!

The boat trip is kindly sponsored by:

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General Information

Language

The official language of the congress is English. No simultaneous translation will be provided.

Internet Access

A laptop with internet access is provided for service during the business hours.

The internet access is kindly provided by

Wireless Internet access is available in case you want to use your own computer. Please check the information board at the registration desk for access details!

Messages

There is a message board close to the congress registration desk.

Congress Bags

Congress bags are kindly provided by

Safety and Security

Please, wear your name badge while in the congress area (access will be denied otherwise). Remove your name badge when leaving the congress area.

The name badges are kindly sponsored by

The lanyards are kindly sponsored by

Ball point pens are kindly provided by

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In case of emergency, please follow directions from the congress staff and chair persons.

General Information

Emergency calls

These are important numbers for the emergency services in Belgium.

National Emergency Services
Medical Service 100
Police 101
Fire Service 100
Pan-European emergency number 112
(calls are free from any mobile cellular of fixed-line telephone)

European SOS 112

The number 112 can be dialled to reach emergency services – medical, fire and police – from anywhere in Europe. This Pan-European emergency number 112 can be called from any telephone (landline, pay phone or mobile cellular phone). Calls are free.

Currency

The Euro is Belgium's currency. For the latest rates, check out www.xe.com.

General Information

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www.pds-europe.com

www.wilresearch.com

Congress Program

10th September, Tuesday

11.30 – 12.15	**Registration Handelsbeurs**
12.15 – 12.30	**Welcome: Introduction to the congress**
	Skin
12.30 – 13.15	**Models of diseased skin (including normal skin)** *Prof. Monika Schäfer-Korting*
13.15 – 14.00	**Reconstructed human skin models: EU validated methods and predictive in vitro tools** *Dr. Christian Pellevoisin*
14.00 – 14.30	**Coffee Break**
14.30 – 15.30	**Key note presentation** **Introduction to dermal studies and the minipig as a model in wound healing** *Dr. Peter Glerup, Dr. Gitte Jeppesen*
15.30 – 16.10	**An in vivo evaluation model of cutaneous tolerance: Development, use, limits and possible refinements** *Dr. Béatrice Gauthier*
16.10 – 16.40	**Short Break**
16.40 – 17.20	**Human skin equivalents: models for cutaneous biology, disease and drug screening** *Prof. Joost Schalkwijk*
17.20 – 18.00	**Bovine corneal opacity and permeability test** *Dr. Sandra De Jonghe and Dr. Ann De Smedt*
19.00 – 21.00	**Reception (Town Hall Ghent).**

Congress Program

11th September, Wednesday

Nervous System

08.30 – 09.10	**Anti-angiogenic therapy of glial brain tumors; lessons learned from human glioma xenograft models** *Prof. Pieter Wesseling*
09.10 – 10.30	**Key note presentation** **Potential and drawbacks of animal and in vitro models to study multiple sclerosis disease mechanisms** *Prof. Jack van Horssen*
10.30 – 11.00	**Poster presentations and coffee break**
11.00 – 11.45	**Using electrophysiological approach to study the CNS white matter; in health and disease** *Dr. Ragnhildur Thora Karadottir*
11.45 – 12.30	**Models of Insertional Mutagenesis following Gene Therapy – a Pathologist's View** *Dr. Jan Klapwijk*
12.30 – 13.30	**Lunch**

IATP session – CNS Evaluation in Rodent Toxicity Studies

13.30 – 13.40	**Introduction by Dr. Robert Sills**
13.40 – 14.25	**Brain microanatomy: cytology, artifacts, neoplastic lesions and utilization of special stains** *Dr. Robert Garman*
14.25 – 15.20	**Overview of non-neoplastic lesions of the nervous system** *Dr. Peter Little*
15.20 – 15.40	**Coffee Break**
15.40 – 16.25	**Use of the NTP revised rodent brain trimming procedure for routine studies in CROs** *Dr. Alys Bradley*
16.25 – 17.10	**Subsite awareness in the NTP CNS protocol** *Dr. Deepa Rao*
17.10 – 17.30	**Panel Discussion – All speakers. Chaired by Dr. Robert Sills**
17.30 – 19.30	**ESTP Annual Assembly**
19.30 – 21.00	**Belgian beer tasting**

Congress Program

12th September, Thursday

LIVER

08.30 – 09.30	**Key note presentation** **In vitro and in vivo prediction of liver toxicity for early selection of drug candidates: cellular models & molecular tools** *Dr. Adrian Roth*
09.30 – 10.15	**FP7 PREDICT-IV consortium – preliminary results from in vitro liver investigations** *Prof. Armin Wolf*
10.15 – 10.45	**Coffee Break**
10.45 – 11.45	**Translational mechanism-based biomarkers for DILI in humans – from molecule to man** *Dr. Daniel Antoine*
11.45 – 12.30	**Drug-induced acute liver damage – liver histopathology** *Prof. Tania Roskams*
12.30 – 13.30	**Lunch**
13.30 – 14.30	**Key note presentation (BSTP-sponsored C. Gopinath lecture)** **DILI, animal models and their use to establish biomarkers for human diagnostic purposes** *Prof. Anja Kipar*
14.30 – 15.15	**Mouse models for cardiometabolic disease with specific focus on non-alcoholic steatohepatitis (NASH)** *Dr. Peter Wielinga*
15.15 – 15.35	**Awards (Novartis publication award, SFPT best poster award, IATP Charles Capen trainee award)**
15.35 – 16.05	**Coffee Break**
16.05 – 16.50	**uPA-SCID mice with a humanized liver: characterization and application** *Dr. Philip Meuleman*
16.50 – 17.50	**Predicting drug-induced hepatotoxicity in zebrafish larvae** *Natalie Mesens, Dr. Aswin Menke*
19.30	**Boat-trip (departure place: Graslei, Gent)**
20.00	**Dinner – Old Fish Market (Oude vismijn, Sint-Veerleplein 5, Gent)**

Congress Program

13th September, Friday

08.30 – 10.45	**Case presentations** Interactive session
10.45 – 11.00	**Coffee Break**
11.00 – 12.00	**INHAND update** **Non-proliferative and proliferative lesions of the digestive tract** *Dr. Thomas Nolte*
12.00 – 12.45	**SEND-INHAND update** **Progress of INHAND and collaboration with the FDA on SEND** *Dr. Susanne Rittinghausen, Dr. Charlotte Keenan*
12.45 – 13.00	**Closing remarks**

Speaker Abstracts

S01: Models of diseased skin (including normal skin)

Monika Schäfer-Korting,
Institute for Pharmacy, Freie Universität Berlin

Animal experiments for the testing of cosmetics and their ingredients have been banned by the EU in its entirety by 2013. In order to allow for testing of potential new active agents as well for the hazard assessment of chemicals alternative approaches have been tested. The result was the development of reconstructed human epidermis (RHE) and respective protocols. Tissue culture makes use of primary human keratinocytes seeded on a supporting membrane and grown in Ca^{2+}-rich medium. Differentiation is induced by air-lift of the culture. Constructs forming a fully stratified human epidermis are available commercially and can be used for the testing of acute local toxicity, i. e. corrosivity, irritancy, phototoxicity (tiered test) according to OECD test guidelines. Moreover, percutaneous absorption studies are possible based on RHE using a validated protocol (1).

Besides the reconstruction of human epidermis, it is also possible to reconstruct full thickness human skin (RHS). Then the keratinocytes are seeded onto human fibroblasts embedded into a collagen gel. RHE and RHS morphology well reflects normal human skin, whereas the stratum corneum lipids are not perfectly formed (2). It is most interesting to note that tight junction proteins are also expressed in RHE (3). The use of RHS is necessary for studies involving dermal fibroblasts. In fact, percutaneous absorption can be investigated in RHE and RHS whereas for biotransformation studies RHS is to be preferred since enzyme expression in keratinocytes and fibroblasts is not identical. Moreover, in order to study influences on dermal function RHS is the test matrix of choice.

For the purpose of an *in vitro* platform in pre-clinical drug development disease models need to be generated. By principle, cells isolated from lesional skin can be used for model building, yet availability of those primary cells is very limited. Use of cells of higher passages for construct building in particular, may result in cell properties no more in line with the respective disease. Therefore, in depth characterization of cell properties is essential. This holds also true, if a defined disease related damage is induced in normal human cells. Defined skin barrier alterations can be realized by knocking down relevant proteins such as corneodesmosin (4) or filaggrin (5, 6) in the normal keratinocytes. Corneodesmosin deficiency results in thickened stratum corneum as to be found in peeling skin disease. Filaggrin loss of function mutations are found in e. g. atopic dermatitis, psoriasis, and ichthyosis vulgaris. A construct based on filaggrin-deficient fibroblasts isolated from lesional skin and normal human keratinocytes is commercially available as psoriasis model (Mattek, MA). Inflammatory responses can be induced by exposure of normal RHS to e. g. TNF-alpha (7, 8) which offers an approach to induce the inflammation seen in psoriasis and atopic dermatitis. These defects alter morphology (8) as well as other relevant functions of human skin, e. g. uptake of xenobiotics (4, 5) and thus the response to irritants (5). Tumor models can be generated by co-cultures of normal keratinocytes and fibroblasts with tumor cell lines. A melanoma model is commercially available (e. g. Melanoma Skin Model, Mattek, MA). A human microvascularized reconstructed skin populated with melanoma cell lines has recently been described. Ability of melanoma cells to cross dermal-epidermal junctions is reported to correlate with the metastatic potential (9). A non-melanoma skin cancer model (10) has been proven suitable for the study of photodynamic therapy.

Speaker Abstracts

Although the inclusion of dendritic cells is of high relevance the reconstruction of human skin populated with dendritic cells (11) is still a major challenge. Thus, current research on testing for sensitization focusses on a tiered test on skin penetration in RHE and activation of dendritic cells (12). RHS as well as reconstructed mucosal and vaginal epithelia can be used for the investigation of Candida infections and the relevance of the cross-talk of leucocytes in the control of infection. Non-specific immune function becomes obvious by TLR-4/2 up-regulation (13, 14).

Moreover, *human-on-a-chip* systems provide the potential to study the cross-talk between organs as well as to replace animal studies even for repeated dose testing (15).

References

1. M. Schäfer-Korting, et al. The use of reconstructed human epidermis for skin absorption testing: Results of the validation study. Altern Lab Anim. 36:161–187 (2008).
2. M. Ponec, et al. Barrier function in reconstructed epidermis and its resemblance to native human skin. Skin Pharmacol Appl Skin Physiol. 14 Suppl 1:63–71 (2001).
3. J.M. Brandner. Tight junctions and tight junction proteins in mammalian epidermis. Eur J Pharm Biopharm. 72:289–294 (2009).
4. V. Oji, et al. Loss of corneodesmosin leads to severe skin barrier defect, pruritus, and atopy: unraveling the peeling skin disease. Am J Hum Genet. 87:274–281 (2010).
5. S. Küchler, et al. Hallmarks of atopic skin mimicked *in vitro* by means of a skin disease model based on FLG knock-down. Altern Lab Anim. 39:471–480 (2011).
6. M. Mildner, et al. Knockdown of filaggrin impairs diffusion barrier function and increases UV sensitivity in a human skin model. J Invest Dermatol. 130:2286–2294 (2010).
7. K.R. Mewes, et al. Elastin expression in a newly developed full-thickness skin equivalent. Skin Pharmacol Physiol. 20:85-95 (2007).
8. G. Weindl, et al. Evaluation of anti-inflammatory and atrophogenic effects of glucocorticoids on reconstructed human skin. Altern Lab Anim. 39:173–187 (2011).
9. L. Gibot, et al. Development of a tridimensional microvascularized human skin substitute to study melanoma biology. Clin Exp Metastasis. 30:83–90 (2013).
10. D. Hoeller-Obrigkeit, et al. Effects of photodynamic therapy evaluated in a novel three-dimensional squamous cell carcinoma organ construct of the skin. Photochem Photobiol. 85:272–278 (2009).
11. N. Bechetoille, et al. A new organotypic model containing dermal-type macrophages. Exp Dermatol. 20:1035–1037 (2011).
12. S.N. Kolle, et al. Strategy for testing skin sensitisation potential without animals. In T. Seidle and H. Spielmann (eds.), AXLR8 Workshop Report, Vol. 3, Berlin, 2013, pp. 201–209.
13. G. Weindl, et al. Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. J Clin Invest. 117:3664–3672 (2007).
14. M. Schaller, et al. Models of oral and vaginal candidiasis based on *in vitro* reconstituted human epithelia. Nat Protoc. 1:2767–2773 (2006).
15. I. Wagner, et al. A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture. Lab Chip (2013).

Speaker Abstracts

So2:Reconstructed human skin models: EU validated methods and predictive in vitro tools

Pellevoisin Christian, PhD
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Reconstructed human skin models: EU validated methods and predictive in vitro tools

In march 2013, the European ban of animal testing for cosmetic ingredients and finished products has dramatically changed the assessment of toxicology in the cosmetic industry. As soon as 1989, L'Oréal decided to stop animal testing for finished products and started research programs to develop more replacement methods for ingredients. Skin engineering, utilized for knowledge research, was a very promising technology for in vitro alternative methods. Based on human primary cells, 3D models better mimic real skin organization than classical 2D cell cultures. They exhibit a functional stratum corneum which allows testing products as in real life conditions and irrespective of the physico-chemical properties (liquid, paste, powder, lipophilic or hydrophilic) of the tested compounds. From the very first model of reconstructed human epidermis (RhE) developed by Prunieras in the eighties, scientists never stopped to invent new models, to industrialize their production and to develop protocols to assess several biological and toxicological endpoints.

RhE are highly versatile systems that can be used both for efficacy and toxicology. Nowadays, RhE allow assessing the efficacy of molecules and formula for a wide range of cosmetic's targets such as skin pigmentation, skin aging, or skin ethnicity. In toxicology, several protocols exist for different endpoints such as skin permeation, phototoxicity, genotoxicity, skin corrosion and irritation, eye irritation etc... In a regulatory context, depending of its status (non validated, pre-validated, validated), an alternative method can be used as a standalone method for full replacement of an animal method or only as a source of supportive information, i. e. part of a weight of evidence justification for example. ECVAM is the European agency which validated scientifically a method prior its regulatory acceptance. Episkin and SkinEthic RHE have been validated by ECVAM respectively in 1998 and 2006 for skin corrosion and in 2007 and 2008 for skin irritation. Since 2004 and 2010, these methods have been integrated in OECD TG431 and 439. The model of humane cornea epithelium (HCE) is engaged in a formal validation as an in vitro method for assessing eye irritation of chemicals.

These methods are now part of an integrated testing strategy used in house for the prediction of ingredients and formula of human safety and efficacy.

Speaker Abstracts

S03: Introduction to dermal studies and the minipig as a model in wound healing

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The skin of pigs and minipigs shows many similarities to human skin. In drug development the most appropriate animal species should always be used for non-clinical safety testing, and for dermal pharmaceuticals it is therefore difficult to justify not to use the pig for these studies.

But also in development of wound care pharmaceuticals and devices, the pig constitutes a much better model than all other animal species. The wound healing process consists of the same phases as in humans and for both humans and pigs, wound contraction occurs quite differently as compared with loose skinned animals. In addition, the sensitivity of the skin of pigs is more similar to human skin, whereas exacerbated reactions are often seen in the rabbit.

This presentation will focus on the use of minipigs in dermal safety studies and in wound healing research for efficacy and safety testing. Scientific as well as regulatory and practical aspects will be discussed and methods used for evaluation of wound healing will be described. In addition to this, abraded skin models for use in regulatory toxicity testing of dermal products and other modified wound healing models will be presented.

The second part of this presentation will give an introduction to the histopathological evaluation of wounds from wound healing studies performed in minipigs. The standard approach used at CiToxLAB Scantox will be presented, including the most commonly employed set of diagnosis and the use of special stains. Examples of full-thickness wounds, split-thickness wounds, necrotic wounds and infected wounds will be given.

Speaker Abstracts

S04: An *in vivo* evaluation model of cutaneous tolerance: development, use, limits and possible refinements

Béatrice Gauthier
Galderma R&D Sophia Antipolis, Preclinical Department, Full Development

When developing a new drug product to treat skin diseases, potent pharmacological agents are needed but in the same time, the formulation needs to be adapted to the clinical conditions properties and to have an acceptable tolerability profile. Thus, in addition to pharmacology skin models and to the toxicology program, animal models of cutaneous tolerance were developed to evaluate and screen the new formulations. In order to build a model as close as possible to human clinical trials, the use of Göttingen minipig was preferred and the studies were designed with reference to the 21-day cumulative irritancy study (phase I clinical trial) and to the Dumas-Scholtz model used for psoriasis clinical evaluation. The minipig minizone model could be used to compare the tolerance profile of drug products and their vehicle placebo before entering in the full development process. When compared to the clinical trials, the minipig was shown to be slightly more sensitive than human probably due to the higher amount of drug product applied but the ranking obtained for the tested products was similar. As limitations for this model, a high inter-individual variability is consistently observed, which implies to have sufficient number of animals on board. In addition, sensitization potential of the tested items needs to be taken into account. The background cutaneous findings in minipig are most generally limited and of no influence on the test results but may, in some rare cases, interfere with the clinical evaluation of skin reactions or modify the tolerability of a test item.

Although mainly based on clinical endpoints, the minipig minizone model may be further refined using non-invasive or invasive methods to evaluate pharmacodynamics biomarkers (genomics and proteomics), to document the histopathology findings or the cutaneous pharmacokinetics.

Speaker Abstracts

S05: Human skin equivalents: models for cutaneous biology, disease and drug screening

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Human reconstructed skin models have been developed and implemented for corrosion and irritation testing, as a replacement for experimental animals. Our lab has designed such models to faithfully mimic normal human skin and oral epithelia to study the biology of these tissues. In addition we have developed models for major inflammatory skin diseases such as psoriasis[1] and atopic dermatitis[2]. These include culture systems based on skin cells only, and systems that study interaction between skin cells and lymphocytes. We have used skin disease models to address basic questions of the biology of these diseases[3], but more recently we showed that they can be used to elucidated the mechanism of drug action[4]. Here I will review the applications of in vitro reconstructed skin beyond corrosion and irritation testing, ranging from biology to disease mechanisms and pharmacology.

1. Tjabringa, G., Bergers, M., van, R. D., de, B. R., Lamme, E., and Schalkwijk, J. (2008). Development and validation of human psoriatic skin equivalents. Am J Pathol 173, 815–823.
2. Kamsteeg, M., Bergers, M., de Boer, R., Zeeuwen, P. L., Hato, S.V., Schalkwijk, J., and Tjabringa, G. S. (2011). Type 2 helper T-cell cytokines induce morphologic and molecular characteristics of atopic dermatitis in human skin equivalent. Am J Pathol 178, 2091–2099.
3. Jansen, P. A., van den Bogaard, E. H., Kersten, F. F., Oostendorp, C., van Vlijmen-Willems, I. M., Oji, V., Traupe, H., Hennies, H. C., Schalkwijk, J., and Zeeuwen, P. L. (2012). Cystatin M/E knockdown by lentiviral delivery of shRNA impairs epidermal morphogenesis of human skin equivalents. Exp Dermatol 21, 889–891.
4. van den Bogaard, E. H., Bergboer, J. G., Vonk-Bergers, M., van Vlijmen-Willems, I. M., Hato, S. V., van der Valk, P. G., Schroder, J. M., Joosten, I., Zeeuwen, P. L., and Schalkwijk, J. (2013). Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis. J Clin Invest. e-pub ahead of print

Speaker Abstracts

So6: The Bovine Cornea Opacity and Permeability test and the added value of histopathology

Ann De Smedt, PhD and Sandra De Jonghe, DVM
Janssen Research & Development (J&J)

In the context of occupational safety, new chemicals used in the pharmaceutical industry, both isolated pharmaceutical intermediates (IPIs) and active pharmaceutical ingredients (APIs), are tested for their potential to cause eye irritation. Such testing is used to ensure that the material is appropriately classified and labeled according to the Globally Harmonised System (GHS).

Although the in vivo Draize rabbit eye test (Draize et al, 1944) has been the reference method for ocular hazard identification for over half a century, this test has been widely criticized for lack of reproducibility, its over prediction and other limitations (Bruner, 1992a; Rowan, 1984; Weil and Scala, 1971). In the light of these concerns and also because of new animal protection laws, several in vitro methods for assessing eye irritation potential have been developed in order to reduce, refine or replace this in vivo assay. Currently only three methods are adopted by the Organisation for Economic Co-operation and Development (OECD) as partial replacements to classify chemicals as inducing serious eye damage (Category 1) and in addition two of these methods were accepted earlier this year for the identification of chemicals not needing a classification for eye irritation. One of these assays is the organotypic Bovine Corneal Opacity and Permeability (BCOP) test method (OECD TG 437), which is used since many years within our company.

The standard BCOP assay (Gautheron et al., 1992) assesses the ocular irritation potential of new chemicals by the use of isolated bovine corneas. Both corneal opacity and dye penetration permeability are measured after treatment (10 minutes or 4 hours depending on physical state of test item). The objective values obtained from both parameters were combined, using a validated classification model, and the resulting in vitro score was compared to a previously established scale of ocular irritancy. This scale is composed of five broad categories: no irritation, mild, moderate, severe and very severe irritation.

Despite the fact the BCOP test is accepted as part of a testing strategy, specific recommendations for improvement were made during a retrospective evaluation of available BCOP data, conducted by the Interagency Co-ordinating Committee for the Validation of Alternative Methods (ICCVAM) and the European Centre for Validation of Alternative Methods (ECVAM) (ICCVAM 2009). One of the recommendations was the addition of histopathology of haematoxylin and eosin (HE)-stained corneal sections by light microscopy, in order to better identify severe irritants. As indicated also by others (Curren et al, 1999, 2000), evaluation of depth and degree of damage could be critical to detect lesions not reflected in direct opacity or fluorescein staining/penetration, certainly for classes of chemicals for which the mode of action cannot easily be predicted.

In this study, we present data obtained from BCOP tests in which corneas were treated with different reference chemicals, covering a broad range of opacity and permeability values, and for which both in vivo Draize data and in vitro BCOP data are available in literature. In addition some internal Janssen compounds were also tested. In addition to the standard BCOP opacity and permeability endpoint, histopathology on HE stained corneal section was also performed for all treated corneas.

Speaker Abstracts

While usually eyes from cattle aging between 12 and 60 months are used in labs performing BCOP assays, our lab routinely uses eyes from cattle less than 12 months of age because of better availability. Due to the fact that the corneal thickness and diameter of these corneas are smaller than that reported from eyes from adult cattle, opacity/permeability values and histopathology analysis will be compared and discussed.

In parallel, solids of which the 20 % formulation was a clear solution were applied both according to the standard protocol of a solid (4 hour treatment) and also according to the liquid protocol (10 minutes treatment and 120 minutes recovery). Opacity/permeability values and the histopathology analysis of both application protocols were compared and evaluated in relation to correct classification for eye irritation.

Histopathology findings were very consistent between corneas within a treatment group. In addition to testing of ocular irritation, the BCOP can sometimes add value in evaluation of the irritant potential of formulations for gavage dosing in rodents. Some examples will be presented.

Curren, R., et al., Optimization of the bovine corneal opacity and permeability assay: histology aids understanding of the EC/HO false negative materials. Altern Lab Anim, 1999. 27: p. 344.
Curren, R., et al., An histological analysis of damage to bovine corneas in vitro by selected ocular toxicants. The Toxicologist, 2000. 54(1): p. 188.
Gautheron, P., et al., Bovine corneal opacity and permeability test: an in vitro assay of ocular irritancy. Fundam Appl Toxicol, 1992. 18(3): p. 442–9.

Speaker Abstracts

S07: Anti-angiogenic therapy of glial brain tumors; lessons learned from human glioma xenograft models.

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Gliomas form a heterogeneous group of tumors of the central nervous system, encompassing many different histological types and malignancy grades. Most gliomas (esp. astrocytic, oligodendroglial, and mixed/oligoastrocytic tumors) are characterized by diffuse infiltrative growth of tumor cells in the surrounding brain. The most malignant (astrocytic) glioma, i. e. glioblastoma, is also by far the most common and is characterized by florid microvascular proliferation and necrosis. Because of this angiogenic response, glioblastomas have since long been considered as good candidates for anti-angiogenic therapy.

Indeed, in different tumor xenograft models inhibition of Vascular Endothelial Growth Factor (VEGF, considered as the main inducer of angiogenesis) resulted in potent anti-tumour effects. Such findings have led to clinical trials in glioma patients with different compounds (both VEGF antibodies and tyrosine kinase inhibitors (TKIs) for VEGF receptors), either as monotherapy or combined with cytostatic agents. Follow-up scans of glioma patients that were treated with bevacizumab often revealed reduced vasogenic brain edema and (esp. in tumors with heterogeneous rather than solid enhancement) reduced contrast enhancement. It soon became clear, however, that normalization of the glioma microvasculature may result in overestimation of the therapeutic efficacy.

Using genotypically and phenotypically relevant, orthotopic models of human gliomas in the mouse brain we have previously shown a dose-dependent effect in the form of increased hypoxia, necrosis, inhibition of glomeruloid microvascular proliferation of vandetanib, a TKI with specificity against VEGFR2, Epidermal Growth Factor Receptor (EGFR) and Rearranged during Transfection/RET in more compact areas, whereas the diffuse infiltrative parts in this model were not notably affected. Moreover, combination treatment with temozolomide and vandetanib had an adverse effect on chemotherapeutic efficacy in this model. Furthermore, in this model vandetanib restored the functionality of the BBB, thereby preventing visibility of tumors using Gd-DTPA-enhanced MRI. A similar effect was found for other AIs (Avastin; Sutent, a TKI of VEGFR, PDGFR, RET, KIT and flt-3). In some experimental glioblastoma models the blockade of vascular changes by anti-VEGF therapy resulted in increased vessel coöption by the tumor.

Unfortunately, in the clinical setting anti-angiogenic therapy for glioblastomas has also not met initial expectations. During the 2013 ASCO meeting in Chicago the results of two large, independent, prospective and randomized trials were reported, the main message being that there was no benefit for overall survival for patients who received anti-angiogenic therapy as part of their treatment for glioblastoma.

Speaker Abstracts

The discrepancy between the effects of angiogenesis inhibitors in some preclinical tumor models and clinical trials warrants a closer look. Part of the explanation may be that in mice carrying rapidly growing subcutaneous tumors, the blood vessels are in a more synchronized stage of development. In patients, tumors are generally more heterogeneous with areas of active angiogenesis co-existing with regions in which the vasculature has already matured. Another very important notion is that diffuse infiltrative tumors may grow without angiogenesis in especially vessel dense-tissues such as lung, liver and brain by incorporating pre-existent vessels.

In conclusion, testing of promising compounds and identification of optimal therapeutic regimens should be performed in genotypically and phenotypically relevant, orthotopic animal models, thereby protecting patients from negative side effects of regimens that are unlikely to provide benefit and saving (sometimes enormous amounts of) for better purposes.

Anti-angiogenic therapy of glial brain tumours

lessons learned from human glioma xenograft models.
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Speaker Abstracts

So8: Potential and drawbacks of animal and in vitro models to study multiple sclerosis disease mechanisms

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General background

Multiple sclerosis (MS) is the most common cause of neurologic disability in young adults, affecting over 2.5 million people worldwide. Initially, most people suffer from defined periods of neurologic deficits followed by complete recovery. This relapsing-remitting phase of the disease gradually evolves into secondary progressive MS in the majority of patients. This advanced stage of the disease is characterized by progressive permanent neurological deficits. A minority of MS patients have a progressive disease course from onset, known as primary progressive MS.

MS histopathology

Examination of MS brain tissue reveals several pathological key features such as inflammation and focal demyelinated lesions scattered throughout the brain and spinal cord. Although traditionally regarded as a pure white matter disease there is significant grey matter involvement, particularly in the progressive stage of the disease. In the first part of my talk I will provide a complementary overview on histopathological features of MS, including sections from different stages of the disease.

What triggers the formation of MS lesions?

New inflammatory white matter lesions in the central nervous system (CNS) evoke clinical relapses, which can be effectively reduced by powerful anti-inflammatory and immunomodulatory drugs. Most of these therapeutics target the adaptive immune system and are aimed at blocking leukocyte infiltration into CNS. Nonetheless, they are unable to prevent worsening of clinical symptoms, suggesting that other pathogenetic disease mechanisms underlie MS progression. In this second part of my talk I will discuss different pathogenetic triggers of MS with a focus on the role of neurodegeneration in MS.

Experimental in vitro and in vivo MS models

The development of in vitro systems to study certain aspects of MS pathogenesis has led to more insight into cellular processes underlying MS lesion formation. Novel techniques have been implemented to isolate and culture relatively pure CNS cell cultures, including oligodendrocytes, microglia, astrocytes and brain endothelial cells. These cell cultures made it feasible to study different aspects of MS lesion formation and progression, such as leukocyte migration across an in vitro human blood-brain barrier or the effects of cerebrospinal fluid on oligodendrocyte function. In addition, multicellular in vitro models have been generated to study processes involved in demyelination and remyelination, such as organotypic brain slice cultures and brain spheroid cultures.

In the last decades, a number of experimental animal models have been developed to mimic certain aspects of MS pathology. Most of these models, including experimental autoimmune encephalomyelitis (EAE) model, are histopathologically characterized by neuroinflammation and demyelination, two main pathological hallmarks of MS. However, no animal model accurately reflects the pathological features that are prominent in the progressive phase of MS, including neurodegeneration. In the last part of my presentation I will describe the most commonly used experimental MS models and discuss their potential and drawbacks.

Speaker Abstracts

S09: Using electrophysiological approach to study the CNS white matter; in health and disease.

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Wellcome Trust/MRC Cambridge Stem Cell Institute & Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom.

The brain's white matter provides an information superhighway that links 100 billion neurons situated in the grey matter. Its function depends on oligodendrocytes wrapping myelin around axons to provide fast neurotransmission, synchronization and maintenance of neuronal function. Despite its importance, the regulation of myelination is unclear. White matter plasticity is increasingly invoked as a mechanism for learning, and destruction of myelination disrupts cognitive and motor function in disease. During development, myelinating oligodendrocytes are generated from oligodendrocyte precursor cells (OPCs), which remain into adult life and comprise about 5 % of the cells in the main proliferating cells present. In demyelinating diseases, such as multiple sclerosis, adult OPCs differentiate into new oligodendrocytes that remyelinate the axons, although this process eventually fails or is incomplete, leading to sustained clinical deficits.

Neurotransmitters, growth factors and electrical activity influence OPCs in normal development and perhaps in disease. By using whole-cell clamping and antibody labelling (Káradóttir & Attwell, 2006) I recently reported that OPCs express NMDA receptor along with the other glutamate ionotropic receptors AMPA and Kainate. OPCs also fall into two groups, either expressing or not expressing voltage-gated Na+ channels. OPCs expressing Na+ channels, fired action potentials and received spontaneous EPSCs and IPSCs, from unmyelinated axons. Generation of human OPCs cells revealed that also human OPCs fall into two classes and fire action potentials.

In an assay in which cortical oligodendrocytes ensheath dorsal root ganglion cells (Wang et. al., 2006) we show that neuregulin and NMDA receptors interact to regulate myelination. Where neuregulin switches myelination from a default programme, that is independent of neuronal activity, to a mechanism that is regulated by glutamate released from active axons.

Using optogenetics to simulate the input from unmyelinated axons to OPCs, we show that the synaptic input regulates proliferation and migration. Moreover, In disease when myelin is damaged, endogenous OPCs are recruited into the lesions to remyelinate the axons. We addressed the role of glutamate and axon-OPCs signalling in the repair process, using an in vivo experimental model of demyelination in the adult rodent and voltage-clamping OPCs recruited to demyelinated lesions. The recruited OPCs, like during development, responded to glutamate and axonal activity and blocking this activity prevented remyelination.

Ischaemia leads to an inward current developing in oligodendrocytes, mediated partly by NMDA and AMPA/kainate receptors (Káradóttir et al., 2005). Where the OPCs with voltage-gated sodium channels were more susceptible to ischemia induced death than the ones lacking voltage-gated sodium channels, consistent with that they have 4fold higher density of NMDA receptors.

These data reveal a function for oligodendrocyte glutamate receptors in myelination and in demyelinating disease; where demyelinated axons remain active and communicate with OPCs through glutamate release during the regenerative process to regulate their remyelination.

Speaker Abstracts

S10: Models of Insertional Mutagenesis following Gene Therapy – a Pathologist's View.

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Discovery and Regulatory Pathology
David Jack Centre for R&D
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After early setbacks and disappointments in the 1990s, great advances have been made with gene therapies (GT) since the start of the millennium. The predominant approach now is to use viral vectors derived from a number of different virus families – vectors which either integrate into patient's DNA or remain episomal. This culminated in the recent approval by EMA of alipogene tiparvovec ("Glybera" from UniQure) for the treatment of lipoprotein lipase deficiency (LPLD), the first GT to have been approved in the Western market. In the same period we have also seen GT trials showing clinical benefit for a number of other conditions including ocular diseases, cancer and Primary Immune Deficiencies (PIDs). In the case of PIDs clear, life-changing clinical improvements have been marred by a significant incidence of leukaemia secondary to what has been termed "insertional mutagenesis" in some, but not all, trials. This presentation will briefly introduce the field of GT, introduce the concept of insertional mutagenesis, it's possible mechanisms and what academia, industry and regulators are doing to try and understand and avoid this serious hazard.

Speaker Abstracts

S11: In vitro and in vivo prediction of liver toxicity for early selection of drug candidates: Cellular Models & Molecular Tools

Adrian Roth
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Selection of drug candidates early on in development has become increasingly important to minimize use of animals and to avoid costly failures of drugs at later stages. While a series of cellular tools have been established to address specific endpoints which can be related to an in vivo liability such as genotoxicity or inhibition of a cardiac channel, in vitro systems to predict and assess organ toxicity have been of limited value so far. The reasons for this are on one side the difficulties in recapitulating in vivo-relevant toxicity on a cell culture level and on the other side that the exact mode of action of often multifactorial organ toxicities are not known and may not be detected by e. g. a simple cytotoxicity assay. To overcome these limitations, researchers have invested into both technologies which are able to capture multiple cellular events simultaneously and on the other hand into in vitro systems which more closely resemble in vivo biology. These aspects have the potential to significantly improve rationale selection of drug candidates. While these approaches offer an enormous potential, the value and acceptance of those new models under real life conditions during drug safety assessment needs to be demonstrated. The presentation aims to give an overview of approaches undertaken in the field of assessment of organ toxicity, in particular liver toxicity, using molecular readouts and physiologically relevant 3D-cell cultures.

Speaker Abstracts

S12: Drug-induced acute liver damage – liver histopathology

Prof. Tania Roskams
Translational Cell & Tissue Research, University of Leuven

Speaker Abstracts

S13: DILI, animal models and their use to establish biomarkers for human diagnostic purposes

Prof. Anja Kipar
Veterinary Pathology, School of Veterinary Science, University of Liverpool, UK

Speaker Abstracts

S14: FP7 PREDICT-IV consortium – preliminary results from in vitro liver investigations

Prof. Armin Wolf, Novartis Institutes for Biomedical Research, Preclinical Safety, Switzerland

Predict-IV represents a consortium of 21 partners from European academia, industry and SMEs with demonstrated excellence in analytical chemistry, biochemistry, cellular models, toxicogenomics, metabolomics, high-content imaging, bioinformatics, statistics, kinetic modeling, toxicology and risk assessment. It is the overall aim of Predict-IV to support the development of better drug testing strategies, which can be used for safety profiling of the most frequently affected target organs of toxicity: liver, kidney and CNS. For each target organ the most appropriate and optimized cellular model was selected. A panel of approximately 10 model compounds, with known *in vivo* outcome in animals and human was selected for each *in vitro* test system. The parallel analyses of dynamic and exposure data after single short-term and multiple long-term treatments, plus a broad spectrum of endpoints were used to rank the compounds according to their specific impaired cellular functions. The current presentation focuses on preliminary results obtained from investigations of rat and human liver in vitro models.

Speaker Abstracts

S15: Translational mechanism-based biomarkers for DILI in humans – from molecule to man

Daniel J Antoine
MRC Centre for Drug Safety Science

Drug-induced liver injury (DILI) represents a significant cause of patient morbidity, mortality and is a major contributor to attrition within drug development. Prediction of clinical DILI remains difficult, particularly in cases characterized by marked inter-individual variation. A lack of sensitivity, specificity and an indirect mechanistic basis of currently used biomarkers of hepatic injury remains a factor for the delayed identification of DILI. Currently, 'Hy's law' represents the regulatory endorsed model to predict serious DILI and the standard for novel DILI biomarkers to surpass. Pre-clinical biomarker identification and validation has been focused on molecular biomarkers such as cytokeratin-18, high mobility group box-1, glutamate dehydrogenase and micoRNA-122 from the perspective of acetaminophen toxicity [Antoine, 2009]. These biomarkers hold translational application to inform the sensitive identification of DILI and its mechanistic basis in man [Starkey-Lewis, 2011. Antoine, 2012]. A number of these biomarkers also provide enhanced prognostic information following acetaminophen overdose [Antoine, 2013]. However, significant challenges such as the characterization of inter- and intra-subject variability, as well as the impact of gender, age, and diurnal variation in healthy volunteers remain regarding these putative biomarkers as well as the utility in idiosyncratic DILI and the prediction of serious DILI from benign elevations in ALT activity. These challenges are the current focus of the Predictive Safety Testing Consortium (PSTC) and the IMI Safer and Faster Evidence based Translation (SAFE-T) consortium. The integrated use of these and qualification strategies will be discussed from a backdrop of imperfect current standards in the context of understanding fundamental hepatology and the response to drugs in model in vitro and in vivo systems.

DJ Antoine et al., 2009 Toxicol Sci. 112(2):521–31
DJ Antoine et al., 2012 J Hepatology. 56:1070–9
DJ Antoine et al., 2013 Hepatology. doi: 10.1002/hep.26294
P Starkey Lewis et al., 2011 Hepatology. 54(5):1767–76

Speaker Abstracts

S16: Mouse models for cardiometabolic disease with specific focus on non-alcoholic steatohepatitis (NASH)

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The incidence of Type 2 Diabetes (T2D) has reached epidemic proportions and more than 350 million patients suffer from this disease. A rapidly growing number of subjects (>750 mio) is at risk and will develop T2D and its life-threatening complications in the near future. Important complications that are associated with T2D are non-alcoholic fatty liver disease (inflamed fatty liver or non-alcoholic steatohepatitis; NASH), renal disease and cardiovascular disease. Current medical treatment appears to be inadequate and does not prevent these complications to happen.

Traditional therapy of subjects at risk of T2D follows strict guidelines and concentrates on optimal glucose control (e. g. by metformin, sulfonylureas or thiazolidinedione). This strategy appears to be inefficient because subjects still develop complications in the liver and vasculature: for instance, about 65 % of all diabetic patients will experience cardiovascular events, despite optimal glucose control.

It is thought that the success of glucose-normalizing strategies is limited because the underlying disease mechanisms remain unaffected leading to the development of severe organ complications (1). The underlying disease mechanisms may be related to metabolic overload. The adipose tissue serves as primary buffer for storage of dietary fat but when the storage capacity of the adipose tissue is exceeded or mitochondrial oxidative capacity is decreased, the adipose tissue becomes a source of inflammation, which is considered metabolic overload. As a consequence, the metabolic and inflammatory pressure on the liver intensifies thereby promoting the development of chronic liver complications like NASH. Hence, there is a need for new approaches that target the pathogenic mechanisms of metabolic overload in pre-diabetic subjects in order to prevent complications of T2D. These approaches should take into account the cross-talk between metabolic organs like adipose tissue and liver.

The development of these multiple-organ complications cannot be studied in humans due to inaccessibility of tissues, duration of pathogenesis and ethical aspects. Therefore robust and translational animal models are needed. This presentation will introduce newly developed translational models of TNO as a suitable models which develops characteristics of the metabolic syndrome and complications comparable to humans (2). Novel interventional strategies aimed at driving forces of these complications, such as interventions in the inflammasome, will be presented.

References

1. Yudkin JS et al, BMJ 2011;343:d7995.
2. Radonjic M, Wielinga PY et al, PLoS ONE 2013; 8(2):e56122.

Speaker Abstracts

S17: uPA-SCID mice with a humanized liver: characterization and applications

Prof. Dr. Philip Meuleman
Center for Vaccinology - Ghent University

Animals have been used for biomedical research already for centuries. Nowadays the mouse is by far the most used vertebrate laboratory animal in the world. However, although laboratory animals have proven their importance for the study of basic biology, (infectious) diseases, drug safety and efficacy, there are several constraints.

For example, certain human infectious diseases cannot be studied in mice, rats or other common laboratory animals because they are naturally resistant to the infectious pathogen. In addition the disease course and outcome could be considerably different in the animal model compared to what is observed in humans. Likewise, the metabolism, pharmacokinetics and toxicological profile of a medicinal compound could be largely influenced by the species in which they are explored. Non-human primates could serve as an alternative but their use is restricted because of ethical and financial restrictions.

About a decade ago we have developed a mouse model with a humanized liver (1). In this model, 2-week old immune deficient (SCID) mice that suffer from a transgene-induced (uPA) liver disease are transplanted with primary human hepatocytes. These human hepatocytes will gradually repopulate the diseased mouse liver up to a point where 60–90 % of mouse liver parenchyma is occupied by healthy, functional human hepatocytes. These mice with humanized liver then become susceptible to infection by human-specific hepatotropic pathogens such as the hepatitis B virus, hepatitis C virus and Plasmodium falciparum (2, 3).

In addition we have shown that the way by which certain compounds are metabolized by the humanized mouse liver closely resembles the metabolism observed in healthy humans; but considerably differs to what is observed in cell cultures and regular mice (4–6).

During my presentation I will provide a functional and morphological characterization of mice with a humanized liver, and will indicate how this model may play an important role in the study of human-type metabolism and drug toxicity.

References

1. Meuleman P, Libbrecht L, De Vos R, de Hemptinne B, Gevaert K, Vandekerckhove J, Roskams T, et al. Morphological and biochemical characterization of a human liver in a uPA-SCID mouse chimera. Hepatology 2005;41:847–856.
2. Meuleman P, Leroux-Roels G. The human liver-uPA-SCID mouse: A model for the evaluation of antiviral compounds against HBV and HCV. Antiviral Res 2008;80:231–238.
3. Sacci JB, Jr., Alam U, Douglas D, Lewis J, Tyrrell DL, Azad AF, Kneteman NM. Plasmodium falciparum infection and exoerythrocytic development in mice with chimeric human livers. International journal for parasitology 2006;36:353–360.
4. Lootens L, Meuleman P, Pozo OJ, Van Eenoo P, Leroux-Roels G, Delbeke FT. uPA+/+-SCID mouse with humanized liver as a model for in vivo metabolism of exogenous steroids: methandienone as a case study. Clin Chem 2009;55:1783–1793.
5. Lootens L, Van Eenoo P, Meuleman P, Leroux-Roels G, Delbeke FT. uPA+/+-SCID mouse with humanized liver as a model for in vivo metabolism of 4-androstene-3,17-dione. Drug Metab Dispos 2009.
6. Pozo OJ, Van Eenoo P, Deventer K, Lootens L, Van Thuyne W, Parr MK, Schanzer W, et al. Detection and characterization of a new metabolite of 17alpha-methyltestosterone. Drug Metab Dispos 2009;37:2153–2162.

Speaker Abstracts

S18: Predicting drug-induced hepatotoxicity in zebrafish larvae

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Zebrafish larvae represent an attractive lower animal model to fill the gap between high throughput *in vitro* cellular assays and conventional preclinical animal testing. The model may, in particular, be useful in predicting human liver toxicity. Drug-induced liver injury (DILI) is poorly predicted by single- cell- based assays, likely due to the lack of the physiological integrations with other cells within the liver. An intact whole liver system of such as one found in a vertebrate animal model such as the zebrafish; could provide added value in a screening strategy for DILI.

The aim of this study was to set up an assay for assessing drug-induced hepatotoxicity in zebrafish larvae. For this purpose, the expression pattern of a liver specific protein in the larval liver was investigated after treatment with reference compounds. Hereto, LFABP10 was chosen as a marker since tissue- specific fatty acid binding proteins were recently suggested as plasma markers for tissue injury (Pelsers et al., 2005). In addition, an investigation of proteomic biomarkers in *in vivo* hepatotoxicity in rats showed that the LFABP proteins were down regulated after acetaminophen exposure, suggesting fabp10 is downregulated during acute hepatocellular necrosis (Yamamoto et al., 2006).

The effect of a number of well characterized reference compounds on fabp10 expression was then investigated. Reference compounds were selected to induce the majority of hepatotoxic phenotypes in humans (cholestasis, steatosis and necrosis) with well described putative mechanisms of toxicity such as inhibition of the bile salt export pump (BSEP), mitochondrial toxicity and reactive metabolite formation.

Hepatotoxic compounds like acetaminophen, amiodarone, tetracycline, nefazodone, and tamoxifen could be identified in the zebrafish larvae by a significant change in the expression pattern of the liver-specific protein *fabp10*. Different expression patterns were observed and compared with the histopathological changes in the liver, both in adults and zebrafish larvae. Whole genome micro-array analysis on the livers or on the whole larvae respectively was included to support the potential histopathological findings.

Speaker Abstracts

S19: INHAND: Non-proliferative and proliferative lesions of the digestive tract

Dr. Thomas Nolte
Boehringer Ingelheim Pharma GmbH & Co. KG

The INHAND nomenclature project is an international effort to create a system of standardized nomenclature and diagnostic criteria for nonproliferative and proliferative histopathology lesions in laboratory animal species. Since its initiation in 2005 the project focused on rats and mice while activities on nonrodent species had just started. INHAND terminology is generally descriptive rather than diagnostic and based on H&E morphology. Further details on the INHAND project have been published by Mann et al. (2012) and can be found on www.goreni.org.

At time of this congress, the nomenclature and diagnostic criteria generated by the INHAND Digestive Tract Organ Working Group were in a final draft status, ready for GESC- and the subsequent membership review. With this presentation the INHAND Digestive Tract Organ Working Group seeks for intense interaction with the scientific community in order to maximize the value of this guide, which will be the future standard for regulatory type toxicity studies (see also the subsequent presentation by PD Dr. Susanne Rittinghausen and Dr. Charlotte Keenan).

The limitation in time of an oral presentation precludes a comprehensive presentation of all diagnostic entities described for the digestive tract, including salivary glands and pancreas. Therefore, the focus will be on (I) diagnostic challenges and (II) significant changes to existing nomenclature systems. Key differential diagnostic criteria will be specifically emphasized.

A major diagnostic challenge in the gastrointestinal tract is the differential diagnosis between infiltration in epithelial malignancies on the one hand and diverticula on the other.

The key criteria for an **adenocarcinoma** of the stomach or intestine are loss of the regular mucosal structure, varying degrees of cellular atypia, many mitotic figures, and invasion of nests and cords of neoplastic cells into lamina propria or deeper layers with loss of basement membrane integrity. Often these tumors elicit a scirrhous response in areas of invasion.

Infiltrative growth by an adenocarcinoma has to be carefully differentiated from diverticula. **Diverticula** represent an extension of mucosal glands or crypts through the muscularis mucosae into the submucosa and further in some cases. The morphology of epithelial lining is variable, ranging from single layer cuboidal or columnar cells to complete mucosa. The epithelium may show features of regeneration like increased basophilia, increased nucleus-to-cytoplasm ratio and gradual loss of polarity, but atypia is minimal at the most. If atypia is more prominent, the modifier *"atypical"* should be added. The modifier *"cystic"* may be used for rounded diverticula that compress surrounding structures. Importantly, diverticula are always sharply demarcated from the surrounding tissue, indicating that basement membrane integrity is always maintained.

Hyperplasia of the gastric or intestinal mucosa may be focal or diffuse. It is confined to the mucosa. The glandular or villous architecture is not altered by the proliferative process itself but in regenerative hyperplasia may have been altered by the initiating event. There is no cellular atypia. Focal penetration by diverticula into the lamina propria or deeper layers may be present, but the basement membrane is always intact. The modifier *"atypical"* should be added if there is cellular atypia and pleomorphism. I such cases, the mucosal structure is often abnormal.

In contrast to the hyperplasia, the **adenoma** of the stomach and intestine grows byond the confinement of the mucosa, projecting as a polypoid, papillary or sessile tumor into the lumen. Adjacent tissue is compressed, if the growth is not exclusively polypoid. The mucosal architecture is distorted. As with the hyperplasia, focal penetration by diverticula

Speaker Abstracts

into the lamina propria or deeper layers may be present, but the basement membrane is always intact. Cellular atypia is usually limited and the polarity maintained.

Gastrointestinal Stromal tumors (GIST) have been described recently in rats and genetically engeneered mice (Fujimoto et al. 2006, Nakai et al. 2008). Using routine H&E staned slides, these tumors are difficult to distinguish from leiomyomas/ leiomyosarcomas or other soft tissue tumors. However, histogenetically they are distinctly different as they develop from the interstitial pacemaker cell, the Cajal cell. Characteristically, these tumors are positive for CD 117, the cytokine receptor encoded by c-kit. A differentiation of GIST from leiomyomas is, therefore, possible only by immunohistochemistry. GIST may exhibit a benign or malignant phenotype.

According to the INHAND principle to base diagostics on H&E morphology it is recommended generally not to differentiate GIST from leiomyomas / leiomyosarcomas or other soft tissue tumors. However, immunohistochemical differentiation of the different tumor types, including GIST, needs to be performed in case of inbalances in the incidence of smooth muscle tumors in a given study.

In the pancreas, ductal metaplasia needs to be carefully differentiated from acinar cell atrophy and acinar cell degranulation.

Acinar cell degranulation is a focal or diffuse process characterized by partial or complete loss of zymogen granules. This leads to small acini lined by cells with reduced size and increased basophilia. Acinar cell degranulation is a common secondary finding in toxicity studies if food consumption is substantially reduced.

Also **acinar cell atrophy** may be focal or diffuse and results in small acini lined by small columnar cells. However, in contrast to acinar cell degranulation the cells lost not only their zymogen granules but also largely the basophilic cytoplams at their basal aspects. What remains are inactive nuclei surrounded by scant cytoplasm. Pyknotic and karyorrhectic nuclei and apoptotic bodies indicate the degenerative character of the process. Atrophic acini may be dilated to duct-like or cyst-like structures which are then lined by cuboidal to flattened epithelium. Due to the reduced acinar volume the intra- and interlobular ducts appear more prominent and more numerous. Further characteristics of acinar cell atrophy are scattered to coalescing interstitial adipocytes and interstitial fibrosis. Transitional structures containing a mixture of normal acinar cells, atrophic acinar cells, and ductal cells may surround areas of atrophy.

As described above, **ductular metaplasia** may result fron acinar atrophy. In fact, it is one feature of acinar atrophy and usually found intermingled with pale atrophic exocrine acini. As such, it is usually present in the acinar compartment rather than in the native ducts. Due to the local and pathogenetic association with atrophy it is usually not diagnosed separately in toxicity studies. These lesions are considered reparative and not preneoplastic. However, the diagnosis of ductular metaplasia may be considered in studies where ductular neoplasms have been induced experimentally.

The entire INHAND nomenclature and diagnostic critieria for the digestive tract, including salivary glands and pancreas, will be available for membership review soon. The Organ Working Groups welcomes any comments and suggestions.

Acknowledgement:

The generation of the INHAND guide for the digestive tract was possible only due to the extraordinary engagement of the members of the Organ Working Group, who are internationally recognized experts in their fields.

Members of the Organ Working Group are:

Patricia Brander-Weber (ESTP), Michael Elwell (STP), Richard Hailey (STP), Thomas Nolte (chair) (ESTP), Cynthia C Shackelford (STP), Andrew Spencer (BSTP), Takuji Tanaka (JSTP), Arun Pandiri (STP), Charles Dangler (STP), Anja Knippel (ESTP), Arlin Rogers (STP), Michael W. Leach (STP), Peter Greaves (BSTP), Ulrich Deschl (ESTP), Jerrold M. Ward (STP)

References

Fujimoto H, Shibutani M, Kuroiwa K, Inoue K, Woo GH, U M, Hirose M (2006). A case report of a spontaneous gastrointestinal stromal tumor. Toxicol Pathol 34: 164–167.
Mann PC, Vahle J, Keenan CM, Baka JF, Bradley AE, Goodman DG, Harada T, Herbert R, Kaufmann W, kellner R, Nolte T, Rittinghausen S, Tanaka T (2012) International Harmonization of Toxicologic Pathology nomenclature: An overview and review of basic principles. Toxicol Pathol 40(45):7–13.
Nakai N, Ishikawa T, Nishitani A, et al. (2008). A mouse model of a human multiple GIST family with KIT-Asp820Tyr mutation generated by a knock-in strategy. J Pathol. ;214:302–311.

Speaker Abstracts

S20: Progress of INHAND and Collaboration with the FDA on SEND

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C. M. Keenan ToxPath Consulting

The INHAND Proposal (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) has been effective since 2005. A Global Editorial Steering Committee (GESC) oversees the project and the development of harmonized terminology for each organ system is the responsibility of the Organ Working Groups (OWG), drawing upon experts from North America, Europe and Japan.

Remarkable progress has been made with six systems published to date – Respiratory, Hepatobiliary, Urinary, Central/ Peripheral Nervous Systems, Male Reproductive and Mammary, Zymbals, Clitoral and Preputial Glands. These documents are available in Toxicologic Pathology as supplements and on a web site – www.goreni.org (member access for scientists working in the field of toxicologic pathology, members of any society of toxicologic pathology or of a regulatory agency). INHAND guides provide diagnostic criteria and guidelines for recording lesions observed in rodent toxicity and carcinogenicity studies. The guides also provide representative photo-micrographs of lesions and information regarding pathogenesis, along with key references.

During 2012, INHAND GESC representatives attended meetings with representatives of the FDA Center for Drug Evaluation and Research (CDER), Clinical Data Interchange Standards Consortium (CDISC), and the National Cancer Institute (NCI) Enterprise Vocabulary Services (EVS) to begin incorporation of INHAND terminology as preferred terminology for SEND (Standard for Exchange of Nonclinical Data) submissions to the FDA. The interest in using the INHAND nomenclature, based on input from industry and government toxicologists as well as information technology specialists, supports that there will be wide acceptance of this nomenclature.

The purpose of this presentation is twofold: 1) to provide a brief historical background and update on the progress of INHAND and 2) to discuss the impact of SEND on toxicologic pathology and the role of INHAND.

Case Presentations

Case 1: Frederic Schorsch; DVM, ECVP Diplomate, Head of Pathology, Bayer Cropscience

CASE TITLE:	Mode of Action and Human Relevance Framework Analysis for Fluopyram-Induced Rodent Liver and Thyroid Tumors
ABSTRACT:	Fluopyram is a broad spectrum pyridylethylamide fungicide. It controls fungi by inhibiting the enzyme succinate dehydrogenase, which is a functional part of the tricarboxylic acid cycle linked to mitochondrial electron transport. Within the registration dossier of new plant pesticides, e.g., herbicides, insecticides, fungicides, a comprehensive evaluation of mammalian toxicity is required to identify potential hazards to human health as well as to derive the reference doses used for human risk assessment. One of the cornerstone studies used for new pesticide registration is the two-species rodent cancer bioassay. The carcinogenic hazard, if any, identified in these studies along with the no observed-adverse-effect levels (NOAELs) are often used to drive the overall human health risk assessment and derive the reference doses. In carcinogenicity studies, fluopyram caused liver tumors in the female rat and thyroid tumors in the male mouse. The proposed mode-of-action (MOA) involves activation of the constitutive androstane receptor (Car) and the pregnane X receptor (Pxr). The mode of action (MOA) for the liver tumors includes the following key events: 1) activation of the Car/Pxr receptors and increased activity of detoxification enzymes, 2) increased hepatocellular proliferation, and 3) increased incidence of altered hepatic foci that progress to liver tumors. The MOA for the thyroid tumors includes the following key events: 1) activation of the Car/Pxr receptors and increased activity of detoxification enzymes, 2) increased thyroxine (T_4) thyroid hormone clearance, 3) increased levels of thyroid stimulating hormone (TSH), and 4) increased follicular cell hyperplasia that process to thyroid tumors. These key events have been evaluated in a series of MOA studies aimed at providing data to perform a weight-of-evidence evaluation using the Bradford-Hill criteria with subsequent application into a Human Relevance Framework (HRF). The conclusion from this evaluation is that fluopyram-induced rodent liver and thyroid tumors via a non-genotoxic MOA that involves the activation of Car and Pxr. The MOA responsible for the tumors seen in fluopyram exposed rodents is similar to that for other Car/Pxr inducers such as phenobarbital (PB). In contrast to rodents, PB exposure in humans does not induce hepatocellular or follicular cell proliferation. This has been shown through extensive human epidemiologic studies where PB exposure comparable to those in rodent studies did not find an increased risk of liver or thyroid cancer. This finding was reinforced during the course of fluopyram mechanistic studies where Pxr-Car knockout mice were refractory to thyroid proliferative effects, whereas wild-type mice did show increased proliferation. Additionally, human primary liver cells in culture exposed to fluopyram showed no increase in proliferation; however it was seen in rat primary liver cells. Due to the epidemiological evidence and lack of proliferation seen in humans, the liver and thyroid tumors induced by Car/Pxr inducers in rodent studies have been identified as not relevant to humans. On this basis, the rodent liver tumors associated with administration of fluopyram would not pose a cancer hazard to humans.

Case Presentations

Case 2: Vanessa Schumacher;

DVM, MS, Dipl. ACVP Principal Scientist/Veterinary Pathologist
Hoffmann-La Roche

CASE TITLE:	*Encephalitozoon cuniculi* in a Rabbit
ABSTRACT:	A 2 year old, female domestic pet rabbit was found lying on its side with dyspnea and acute head tilt. The rabbit died shortly afterwards and submitted to necropsy at the University of Bern Institute for Animal Pathology. Gross findings included multiple, well delineated dark brown discolorations in the brain parenchyma (hemorrhages), which were most extensive in the thalamic region. Other macroscopic lesions included bilateral depressions on the renal surface extending into the cortex (chronic infarcts) and mild pulmonary edema. Microscopically, there was multifocal neutrophilic vasculitis and fibrinoid necrosis in the brain, along with lymphocytic meningoencephalitis and multiple granulomas containing organisms morphologically consistent with the microsporidian parasite *E. cuniculi*. In addition, there was multifocal hepatic necrosis with haemorrhage, interstitial nephritis with tubular necrosis, and multifocal vasculitis in the lung. This case was unusual in that there was extensive haemorrhage and vasculitis in the brain, alongside the granulomatous and lymphocytic meningoencephalitis typically seen in this disease. Herpesvirus was considered a differential diagnosis for the vasculitis and haemorrhage and was ruled out by PCR.
Label on histoslides	S13-5950
ANIMAL(S):	
Species, breed	Domestic Rabbit, *Oryctolagus cuniculus*
Sex	Female
Age	2 years
Study type	None
Treatment	Not applicable
Clinical findings	Acute head tilt to the right, found lying on the side. Temperature 39 °C, dyspnea.
Organ(s)	Brain
Gross finding(s)	Gross findings included multiple, well delineated dark brown discolorations in the brain parenchyma (hemorrhages), most extensive in the thalamic region. There were multiple sunken areas along the surface of both kidneys (chronic infarcts) and there was mild pulmonary edema.
Staining	HE, Gram Stain
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Severe, multifocal, necrohemorrhagic meningoencephalitis with vasculitis and multiple granulomas containing spores consistent with *Encephalitozoon cuniculi*.

Case Presentations

CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	Microscopically in the brain, multiple medium to large blood vessels including those in the choroid plexus were brightly eosinophilic with loss of structural detail (fibrinoid necrosis) and infiltration of heterophils (vasculitis), surrounded by extensive areas of hemorrhage. Adjacent nervous tissue was necrotic. There were multifocal perivascular cuffs consisting of moderate numbers of lymphocytes and few macrophages. Randomly scattered throughout the parenchyma, there were several granulomas containing spores consistent with *Encephalitozoon cuniculi*. These spores were approximately 1.5 x 2.5 µm in size and stained Gram positive with a clear posterior vacuole. There was diffuse gliosis and the meninges were expanded by small numbers of lymphocytes and macrophages. Neurologic signs are a common disease manifestation in rabbits. The most common causes include bacterial disease such as pasteurellosis, encephalitozoonosis, trauma, heat stress, toxemia and cerebral larval migrans. *E. cuniculi* is a microsporidian obligate intracellular organism that has features of both fungi and protozoa. Typical microscopic lesions of *E. cuniculi* in the brain of rabbits are multifocal necrosis, granulomas, perivascular lymphocytic cuffs and meningitis with intralesional spores measuring 1.5 x 2.5 µm in size. Spores are Gram positive and are green birefringent when viewed under polarized light. In this case the typical lesions of lymphocytic and granulomatous meningoencephalitis were accompanied by extensive vasculitis and hemorrhage, which are not commonly described in rabbits. Interestingly, in a study of encephalitozoonosis in squirrel monkeys, vasculitis in the brain was a common finding. Herpes virus infection was considered in this case due to the presence of vasculitis and hemorrhage, and was ruled out by PCR using Herpesvirus consensus primers.
LITERATURE:	Deeb BJ, Carpenter JW. Rabbits: Neurologic and Mukuloskeletal Diseases. In: Quesenberry KE and Carpenter JW. Ferrets, Rabbits and Rodents: Clinical Medicine and Surgery, Second edition. St. Louis, USA: Saunders; 2004: 203–210 Künzel F, Joachim A. 2010. Encephalitozoonosis in Rabbits. Parasitology Research. 106:299–309. VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose T. 1996. Detection and analysis of diverse herpesviral species by consensus primer PCR. Journal of Clinical Microbiology. 34(7): 1666–1671. Wasson K and Peper RL. 2000. Mammalian Microsporidiosis. Veterinary Pathology. 37:113–128. Zeman DH, Baskin GB. 1985. Encephalitozoonosis in Squirrel Monkeys. Veterinary Pathology. 22:24–31.

Case Presentations

Case 3: Marjolein Van Heerden; DVM, Diplomate ECVP;
Bhanu Singh; DVM, Diplomate ACVP
Drug Safety Sciences: Toxicology/Pathology Janssen Research & Development

CASE TITLE:	Morphological Phenotyping of genetically obese (ob/ob) mouse.
ABSTRACT:	The case presentation documents phenotypic characteristics of ob/ob mice, an animal model of type II diabetes. ob/ob mice are deficient in leptin, a major hormonal product of the adipocyte that regulates appetite and reproductive function. Selected tissues (kidneys, liver, lungs, spleen, pancreas, GI tract, lymphnodes, testes, epididymides and bone) were collected from 4 months old male ob/ob mice from the control group of a study. These tissues were processed for histopathological examination. Microscopically, the tissues were evaluated and morphological features were compared with age matched wild type mice. Relevant histological changes were observed in various tissues (liver, pancreas, adrenal gland, femur, testes and epididymides). These characteristics are related to the genotype of the ob/ob mice, and should be considered when ob/ob mice are used in experimental settings.
Label on histoslides	N/A
ANIMAL(S):	
Species, breed	Mouse
Sex	Male
Age	4 Months
Study type	Exploratory
Treatment	None (control)
Clinical findings	Obese mice, hyperglycemia and hyperinsulinemia
Organ(s)	Pancreas
Gross finding(s)	none
Staining	HE
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Pancreas : Islet Cell hyperplasia
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	In addition to islet cell hyperplasia, following microscopic findings were observed: lipidosis in the liver, cortical hypertrophy in the adrenal glands and physeal dysplasia in femur, increased germ cell degeneration and Sertoli cell vacuolization in testes along with germ cell debris in epididymis. These changes in various tissues were related to genotype of the mice.

Case Presentations

LITERATURE:	Lindström P (2007). The physiology of obese-hyperglycemic mice [ob/ob mice]. *Scientific World Journal* 7: 666–85. Bhat, G. K. et al. (2006). Influence of a leptin deficiency on testicular morphology, germ cell apoptosis, and expression levels of apoptosis-related genes in the mouse. J Androl 27: 302–310. Kishida et al. (2005) Leptin regulates chondrocyte differentiation and matrix maturation during endochondral ossification Bone, 37: 607–621.
ADDITIONAL COMMENTS:	

Case Presentations

Case 4: Eva Tibaldi; PhD, Cesare Maltoni Cancer Research Centre Ramazzini Institute Bologna, Italy

CASE TITLE:	Pancreatic Islet cell tumours observed in Sprague_Dawley rats treated with low dose Gamma-Radiation
ABSTRACT:	1,046 Sprague Dawley rats (524 males and 522 females) were treated with 0,1 Gy one off (dose of the same size of a total body CAT) at 6 weeks of age. Animals were observed for the whole life span. In females a statistically significant increase of Islet cell carcinomas was observed (11 cases/522 animals corresponding to 2.1%) when compared to the control, which had the same number of animals, where no carcinoma was observed. In our historical control the incidence of this tumor is 0.04%. In the same group 46 adenomas of the Islet cell were observed (8.8%) very close to the incidence of the control group (7.6%). Some examples of this benign and malignant tumors are presented; RITA nomenclature was used. In our opinion it is noteworthy that carcinomas were only in 2 cases sporadic; others 9 cases were always accompanied by one or more brothers or sisters with Islet cell adenomas, leading to the assumption that pancreas tumors are associated to familiar susceptibility. Paraffin blocks are available for further investigation.
Label on histoslides	Case 1: BT 1R 4094
ANIMAL(S):	
Species, breed	Sprague-Dawley rats
Sex	Female
Age	127 weeks at death
Study type	Long-term carcinogenesis bioassay
Treatment	Gamma radiation at 0.1Gy
Clinical findings	–
Organ(s)	Pancreas
Gross finding(s)	Nodule 0.5 cm diameter, brownish coloration and soft
Staining	Ematoxilin-Eosin
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Islet cell Carcinoma
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	Fibrous capsules more prominent are present. Histological features similar to benign tumors, however, the tumor cells are polyhedral, fusiform or slightly pleomorphic. Mitotic activity is moderate. Growth pattern includes sheets, nests and ribbons. Local invasion of capsule and adjacent pancreas and distant spread of islet tumor cells are present. Because the expansive growth occurs the remnants of primitive fibrous capsule are embedded in the mass.

Case Presentations

LITERATURE:	RITA nomenclature at website: http://www.goreni.org/ Mega-experiments on the carcinogenicity of G-radiation on Sprague-Dawley rats at the Cancer Research Centre of the European Ramazzini Foundation of Oncology and Environmental Sciences: plan and report of early results on mammary carcinogenesis. Soffritti M, Belpoggi F, Minardi F, Bua L, Maltoni C.. Eur J Oncol 4:509–522, 1999
ADDITIONAL COMMENTS:	–

Case Presentations

Case 5: Tania Carvalho; DVM, PhD, Instituto de medicina molecular, falcudade de medicina da universidade de lisboa

CASE TITLE:	Meningeal leukemia is associated with distinct leukemic cell phenotype in a murine xenotransplant model of B-cell acute lymphoblastic leukemia
ABSTRACT:	To address the dynamics and mechanistic basis of nervous system leukemia, sub-lethally irradiated SCID mice xenografts of B cell (B-ALL, 697 cell line) acute lymphoblastic leukemia were used. These mice consistently develop rapidly progressing fatal leukemia, characterized by early colonization of bone marrow (in appendicular/long bones; and axial bones – skull and vertebral bodies), and by meningeal infiltration. Histological analysis of leukemic cells infiltrating the dorsal root nerves, ganglia and leptomeninges showed cells that are invariably larger than their bone marrow infiltrating counterpart, and immunohistochemical analysis showed phenotypic heterogeneity of this population, frequently with loss of Pax5, vimentin and TdT expression; while bone marrow infiltrating leukemic cells maintain the expression of these markers throughout the disease time-course, similarly to B-ALL cells in vitro. This reversion of B cell commitment upon loss of Pax5 and TdT is accompanied by CD19, CD33, CXCR4 and Flt-3 down-regulation, and CX3CR1 and fractalkine (CX3CL1) overexpression, assessed by RQ-RT-PCR in cerebrospinal fluid (CSF) infiltrating B-ALL cells, collected from the *cisterna magna* of late stage leukemic mice. To search for lineage shifts/abnormal patterns of expression of myeloid, T or B lymphoid–associated antigens in these B-ALL cells, a panel of cluster differentiation markers was studied (CD2, CD3, CD10, CD11c, CD20, CD34, CD45, CD79a and CD117), which were all negative in both B-ALL cell subsets, bone marrow and CNS infiltrating.
Label on histoslides	LEUKEMIA CNS
ANIMAL(S):	**Mouse 1–5**
Species, breed	Mouse, BLABc/scid
Sex	Male
Age	8 weeks
Study type	Cancer
Treatment	n. a.
Clinical findings	Leukemia meningeal invasion with loss of B-cell markers
Organ(s)	CNS and PNS (brain and spinal cord)
Gross finding(s)	n. a.
Staining	H&E and immunohistochemical panel (hematopietic markers)
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Meningeal leukemia with loss of B-cell markers

Case Presentations

CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	Meningeal-infiltrating leukemic cells loose the B-cell markers Pax5 and Tdt, and down-regulate CD19, CD33, CXCR4 and Flt-3. Pax5 expression is continuously required to maintain B cell lineage commitment, and its loss was seen to convert committed pro-B cells into hematopoietic progenitors with multi-lineage potential (Mikkola et al., 2002). When analyzing the immunophenotypic changes in B-ALL between diagnosis and relapse, using broad antibody panels, another study refers abnormal expression patterns of TdT, with loss of expression at relapse, as one of the most common aberrancies (Chen et al., 2007). Furthermore, there are several reports concerning immunophenotypic and cytogenetic changes in ALL at relapse (Obro et al., 2011; Raimondi et al., 1993). The underlying mechanism or biological consequences of these phenotypic changes remain yet to be clarified.
LITERATURE:	Chen, W., N.J. Karandikar, R.W. McKenna, and S.H. Kroft. 2007. Stability of Leukemia-Associated Immunophenotypes in Precursor B-Lymphoblastic Leukemia/Lymphoma. *American Journal of Clinical Pathology*. 127:39–46. Mikkola, I., B. Heavey, M. Horcher, and M. Busslinger. 2002. Reversion of B Cell Commitment upon Loss of Pax5 Expression. *Science*. 297:110–113. Obro, N.F., H.V. Marquart, H.O. Madsen, L.P. Ryder, M.K. Andersen, B. Lausen, B.K. Albertsen, P.S. Wehner, J. Helgestad, and K. Schmiegelow. 2011. Immunophenotype-defined sub-populations are common at diagnosis in childhood B-cell precursor acute lymphoblastic leukemia. *Leukemia*. 25:1652–7. Raimondi, S.C., C.H. Pui, D.R. Head, G.K. Rivera, and F.G. Behm. 1993. Cytogenetically different leukemic clones at relapse of childhood acute lymphoblastic leukemia. *Blood*. 82:576–580.
ADDITIONAL COMMENTS:	

Case Presentations

Case 6: Monique Y. Wells;

V.M.D. M.S. Toxicology/Pathology Services Inc. Diplomate, A.C.V.P., E.C.V.P, A.B.T.

CASE TITLE:	An Unusual Finding in the Larynx of Wistar Rats from a Short-term Oral Toxicity Study
ABSTRACT:	Papillary folds were observed in the larynx of male and female control Wistar rats from a 4-week toxicity study. This finding consisted of one to several fronds lined with low cuboidal to cuboidal epithelium that projected into the lumen of the larynx. It was restricted to the ventral pouch epithelium and, more rarely, to the epithelium overlying the U cartilages. Similar lesions were observed in animals receiving the test compound without a clear relationship to dose. Because the incidence of this finding was increased in recovery control animals compared to controls, it may represent an unusual variant of normal histology of the ventral pouch of the rat larynx. Given that the larynx is rarely evaluated in oral toxicity studies, few to no historical control data are likely to be available for this finding. Possible causes include the presence of refluxed gavage material in the ventral pouch and trauma from insertion of the gavage tube.
Label on histoslides	Photomicrographs only; no slides are available
ANIMAL(S):	
Species, breed	Wistar rat
Sex	Male and female
Age	~12 weeks
Study type	4-week oral toxicity study
Treatment	Control animals
Clinical findings	Reflux
Organ(s)	larynx
Gross finding(s)	none
Staining	H&E
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Papillary folds, mucosa, larynx
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	The finding consisted of one to several fronds lined with low cuboidal to cuboidal epithelium that projected into the lumen of the larynx. It was restricted to the ventral pouch epithelium and, more rarely, to the epithelium overlying the U cartilages. Because the incidence of this finding was increased in recovery control animals compared to controls, it may represent an unusual variant of normal histology of the ventral pouch of the rat larynx.

Case Presentations

LITERATURE:	none
ADDITIONAL COMMENTS:	Given that the larynx is rarely evaluated in oral toxicity studies, few to no historical control data are likely to be available for this finding. Possible causes include the presence of refluxed gavage material in the ventral pouch and trauma from insertion of the gavage tube.

Case Presentations

Case 7: Torrie A. Crabbs; DVM, DACVP Experimental Pathology Laboratories, Inc. Research Triangle Park, NC, USA

CASE TITLE:	Lung Lesions in Control Rats from Gavage Studies
ABSTRACT:	While it is well known that gavage accidents occur, references describing lung lesions related to gavage procedures or associated with gavage administration are lacking. Lung slides from male and female control Fischer 344 rats from two chronic gavage studies (corn oil = vehicle control), one chronic water study, and one chronic feed study (50 animals/sex/study) were evaluated for lung changes to determine if there were any differential effects associated with route of administration. While spontaneous alveolar histiocytosis was present in approximately 50–60% of rats, irrespective of administration route, rats from the gavage studies exhibited distinct centriacinar lesions that were not noted in the rats from the other two studies. These lesions consisted of a chronic active inflammatory infiltrate that was centered on the bronchiolo-alveolar junction, and was predominately composed of foamy macrophages admixed with lesser numbers of neutrophils and lymphocytes. Bronchiolar metaplasia (bronchiolization) and type II alveolar epithelial hyperplasia were also frequently present. Numerous pale yellow homogenous droplets that stained positively for Sudan Black were commonly present within the alveolar spaces of the rats from these studies. Given that centriacinar lesions are often associated with inhaled intoxicants and that corn oil served as the vehicle control in these studies, the droplets likely represent aspirated corn oil. Given the lack of significant information on lung lesions in control animals, these findings will serve as a useful addition to the literature and add insight to possible pathogeneses for the presence of lung lesions in animals from non-inhalation studies.
Label on histoslides	
ANIMAL(S):	
Species, breed	F344/N
Sex	Male
Age	2 yrs.
Study type	2 yr. chronic carcinogenicity study
Treatment	Oral Gavage; Vehicle control = corn oil
Clinical findings	None
Organ(s)	Lung
Gross finding(s)	None
Staining	H&E
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Lung – Chronic active inflammation with bronchiolar metaplasia and type II epithelial hyperplasia Lung – Accumulation, Corn Oil

Case Presentations

CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	Rats from the gavage studies exhibited distinct centriacinar lesions that were generally lacking in the rats from the feed or water studies. These lesions consisted of aggregates of foamy macrophages admixed with lesser numbers of neutrophils and lymphocytes that were consistently centered on the junction of the terminal bronchioles and alveolar ducts (centriacinar). These lesions were frequently associated with bronchiolar metaplasia and/or type II alveolar epithelial hyperplasia. In general, all lesions were minimal to mild. Numerous pale yellow homogenous droplets were present within the alveolar spaces of many of the rats from the gavage studies, but were lacking in all of the rats from the feed and water studies. These droplets were presumed to be droplets of corn oil, the vehicle control. This was confirmed by positive staining of the droplets for Sudan Black. The Sudan Black staining was performed on the FFPE tissues. No Sudan Black positive droplets were present in any of the animals from the feed or water study, though there was positive staining of peribronchiolar fat (positive internal control). While it is possible that respiratory tract lesions in non-inhalation studies represent a systemic effect of the test compound, additional mechanisms for exposure must be considered, especially in the case of oral gavage studies where technical gavage errors, gavage-related reflux, spontaneous reflux, and accidental aspiration can all occur.
LITERATURE:	Arantes-Rodriques R, Henriques A, Pinto-Leite R, Faustino-Rocha A, Pinho-Oliveira J, Teixeira-Guedes C, Seixas F, Gama A, Colca B, Colca A, and Oliveira PA. (2012). The effects of repeated oral gavage on the health of CD-1 mice. *Lab Anim.* 41(5):129–134. Damsch S, Eichenbaum G, Tonelli A, Lammens L, Van Den Bulck K, Feyen B, Vandenberghe J, Megens A, Knight E, and Kelley M. (2011). Gavage-related reflux in rats: Identification, pathogenesis, and toxicologic implications (Review). *Toxicol Pathol.* 39:348–360. Damsch A, Eichenbaum G, Looszova A, Lammens L, Feyen B, Van Den Bulck K, Knight E, Kelley M, and Tonelli A. (2011). Unexpected nasal changes in rats related to reflux after gavage dosing. *Toxicol Pathol.* 39:337–347. Sells DM, Brix AE, Nyska A, Jokinen MP, Orzech DP, and Walker NJ. (2007). Respiratory tract lesions in noninhalation studies. *Toxicol Pathol.* 35:170–177.

Case Presentations

ADDITIONAL COMMENTS:	Oral gavage is a common method for administering test compounds to laboratory animals in pharmacological and toxicological studies. It enables direct delivery of the test substance into the stomach, ensuring precise control of dosage and timing of delivery. However, complications with this procedure are not uncommon and the skills of an experienced technician are required. Esophageal irritation and/or perforation with intrathoracic deposition of gavage material, inadvertent tracheal administration, or direct instillation of gavage material into the lung proper can all occur. These types of accidents often result in a marked inflammatory response causing respiratory compromise and, in severe cases, early death. However, not all gavage related accidents result in such a profound effect. During the oral gavage procedure, a limited amount of test article often adheres to the tip of the needle. Aspiration of small amounts of test article likely occurs much more often than suspected, resulting in deposition of varying amounts of the test article (and vehicle control) within the airways. Depending on the amount, concentration, viscosity, and irritancy of the substances, these animals may remain subclinical with minimal to no histologic changes; however, in some cases lesions can become more evident and/or begin to exhibit a dose response.

Case Presentations

Case 8: Alessandro Piaia; Medico Veterinario, ECVP dipl. Novartis Pharma AG, Switzerland

CASE TITLE:	**The elusive Globule Leukocyte, a poorly-characterized cell population of leukocytes**
ABSTRACT:	Globule leukocytes are one of the few cells in the body with an unclear origin and function. They remain a matter of controversy in the literature, despite their striking morphology and relatively easy recognition within the mucous membranes of many vertebrates, including humans. In the rat, globule leukocytes are commonly found in the respiratory airways (upper trachea and larynx) and in the gastrointestinal tract (gastric glandular mucosa in the vicinity of the limiting ridge). They sit between mucosal epithelial cells and display specific histological characteristics (large eosinophilic cytoplasmic granules and lymphocyte-like nucleus) which make them easily recognized. In many histochemical and/or immunohistochemical methods performed to understand their nature, different conclusions were made; they have been related to either mast cells or to large granular lymphocytes. In toxicologic pathology, their number may be increased or decreased by treatment. Both alterations in numbers were observed in our facility: an increase in the gastric mucosa and a decrease in the laryngotracheal mucosa with two different compounds in two different studies. These observations may shed some lights on the origin of the globule leukocytes.
Label on histoslides	
ANIMAL(S):	**Rat**
Species, breed	Han Wistar
Sex	
Age	2 to 5 months
Study type	Study 1: 13-week oral toxcitiy study in Han Wistar rat Study 2: 10-day DRF inhalation study in Han Wistar rat
Treatment	Oral or inhalation studies
Clinical findings	NA
Organ(s)	Study 1: – Stomach Study 2: – Trachea
Gross finding(s)	None
Staining	H/E
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Study 1 Globule leukocyte increased, gastric glandular mucosa, Study 2 Globule leukocyte decreased/absent, tracheal mucosa

Case Presentations

CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	Study 1 Rat, 13 week oral study In the gastric glandular mucosa, especially adjacent to the limiting ridge, submucosal eosinophilic inflammation was present, accompanied by increased number of globule leukocytes in the overlying glandular mucosa. Study 2 Rat 10-day DRF inhalation study In the rostral trachea, globule leukocytes were decreased to absent in the mucosa of treated animals, with the epithelium not showing other signs of toxicity or irritation.
LITERATURE:	1) S.O. Akpavie and H.M. Pirie 1989 "The Globule Leukocyte: Morphology, Origin, Function and Fate, a Review" Anat. Histol. Embryol. 18: 87–95 2) E.K. Tam et al 1988 "Globule leukocytes and mast cells in the rat trachea: their number, distribution, and response to compound 48/80 and dexamethasone" Anat. Embryol 178: 107–118. 3) L. Fan and S. Iseki 1999 "Immunohistochemical localization of vascular endothelial growth factor in the globule leukocyte / mucosal mast cells of the rat respiratory and digestive tract" Histochem Cell Biol 111:13–21 4) Y. Ikeada, S. Yamashina 1993 "Developmental changes in intestinal globule leukocytes of normal rats" Cell Tissue Res. 273: 447–455.
ADDITIONAL COMMENTS:	The presentation will be focusing on the cell population, the changes in two different studies, and an overview on the current literature of these cells in the rat.

Case Presentations

Case 9: Ursula Junker; Veterinary Pathologist, Preclinical Safety, Novartis Pharma AG

CASE TITLE:	**Adenocarcinoma of the salivary gland in a young female Balb/c mouse** **Authors: Junker U, Kittel B, Theil D** *(final list may be extended depending on additional contributions)*
ABSTRACT:	A small tumor was detected as an incidental finding in the sublingual salivary gland of a young female Balb/c mouse, age 9 weeks, which was in an efficacy, tolerability, PK/PD experiment and received a test compound (pharmaceutical) for 8 days. The tumor was preliminary diagnosed as adenocarcinoma based on the solid appearance, cellular pleomorphism and not well defined demarcation to the normal acinar tissue *(further immunohistochemical characterization is currently ongoing and will be included in the final presentation)*. Spontaneous tumors of the salivary gland are uncommon in the strains of rodents commonly used in toxicity studies. The highest incidence of myoepitheliomas has been reported in the Balb/c strain. Salivary gland tumors may be confused with mammary tumors due to the proximity of mammary tissue, but in the present case the tumor is clearly located within the salivary gland and was considered to be of spontaneous origin, since treatment duration of 8 days is too short for induction or promotion of a neoplasm.
Label on histoslides	
ANIMAL(S):	
Species, breed	Mouse, Balb/c
Sex	Female
Age	9 weeks
Study type	Efficacy, PK/PD, tolerability study
Treatment	Pharmaceutical, low dose for 8 days
Clinical findings	None
Organ(s)	Sublingual salivary gland, unilateral
Gross finding(s)	None
Staining	H&E
CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	Adenocarcinoma, sublingual salivary gland

Case Presentations

CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	The tumor is of small size (approx. 1 mm in diameter), composed of pleomorphic cells with various amounts of cytoplasm forming a solid pattern of growth. Nuclei are large, round to oval in shape and often densely clustered. Structures resembling vaguely tubular or acinar structures are only rarely observed in the periphery of the tumor. Mitotic figures are present, but no necrosis. There is no evidence of secretory granules within the tumor cells. A thin fibrous capsule is present around the tumor, but nest of cells grow out of the capsule into the surrounding normal glandular tissue *(further immunohistochemical characterization is currently ongoing and will be included in the final presentation)*.
LITERATURE:	International Classification of Rodent Tumors, The Mouse Gastrointestinal Tract. Springer, Berlin Heidelberg New York 2001, p 28 Bundza A, Charlton KM, Becker SA (1989). Adenocarcinoma of the salivary gland in a Swiss White mouse. Can J Vet Res 53: 363–365 Simons AL, Lu P, Gibson-Corley KN, Robinson RA, Meyerholz DK, Colgan JD (2013). The Justy mutant mouse strain produces a spontaneous murine model of salivary gland cancer with myoepithelial and basal cell differentiation. Laboratory Investigation 93: 711–719 Sundberg CA, Hanson DR, Roor DR, Brown KS, Edigan HG (1991). Myoepitheliomas in inbred laboratory mice. Vet Pathol 28: 313–323
ADDITIONAL COMMENTS:	

Case Presentations

Case 10: Franck Chanut; Pathology Director, ECVP Dipl. GlaxoSmithKline

CASE TITLE:	Have you seen this? An unusual glomerular change
ABSTRACT:	This case presentation will describe an efficacy study performed in Sprague-Dawley rats given a new peptide with the following study design: Group 1 male rats (n = 7) were given the vehicle subcutaneously for 28 days Group 2 male rats (n = 7) were given the test-article subcutaneously for 7 days Group 3 male rats (n = 7) were given the test-article subcutaneously for 28 days Test article-related changes were observed in the kidneys of males given the test article for 28 days. The kidneys had multifocal areas of tubular degeneration with tubular dilatation, small clusters of tubular proteinaceous casts and occasional tubular cellular casts. Glomeruli had prominent parietal epithelium, numerous adhesions of the parietal and visceral epithelium, mitotic figures and small clusters of eosinophilic granules. Occasional glomeruli had small amorphous, pale accumulations of eosinophilic material. Electron microscopy revealed large numbers of electron-dense lysosomes in the podocytes. The granules observed first in H&E appeared to contain the drug as demonstrated by an IHC using an anti-drug antibody. The nephropathy observed could be secondary to the protein overload, but a direct pharmacologic effect can not be excluded. The glomerular change has been described in puromycin aminonucleoside and hyperalbuminaemic induced proteinurias in the female Wistar rat (Lawrence and Brewer 1983). Podocytes have a phagocytic role and new evidence suggests the existence of an active transport mechanism in the podocyte to remove immunoglobulins accumulated at the filtration barrier (Kumagai *et al* 2012)
Label on histoslides	N/A
ANIMAL(S):	
Species, breed	SD rats
Sex	Males
Age	8–10 weeks
Study type	Efficacy Study
Treatment	Undisclosed
Clinical findings	None
Organ(s)	Kidneys
Gross finding(s)	None
Staining	H&E

Case Presentations

CONTRIBUTOR'S MORPHOLOGIC DIAGNOSIS:	glomerulonephropathy with intra-glomerular eosinophilic droplets.
CONTRIBUTOR'S DESCRIPTION AND COMMENTS:	/
LITERATURE:	Lawrence GM, Brewer DB. **A morphometric, biochemical and histochemical comparison of puromycin aminonucleoside and hyperalbuminaemic induced proteinurias in the female Wistar rat.** Journal of pathology, 1983 139(2):115–40. Kumagai T, Mouawad F, Takano T. **Pathogenesis of common glomerular diseases – role of the podocyte cytoskeleton.** Cell Health and Cytoskeleton 2012:4 103–118
ADDITIONAL COMMENTS:	"All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals"

Poster Abstracts

P01: Non-invasive monitoring of Hepatic Stellate Cell activation and potential proliferation

Ashwini Ketkar- Atre[1], Marjolein van Heerden[2], Anja Kimpe[2], Erio Barale[2], Ann Van Santvoort[1], Uwe Himmelreich[1], An Vynckier[2]

[1]Biomedical MRI, MoSAIC, Department of Imaging and Pathology, KU Leuven, Leuven, Belgium
[2]Department of Drug Safety Sciences, Janssen Research & Development, Beerse, Belgium

Stimulation of hepatic stellate cells (HSCs) results in activation and transformation to proliferative, fibrogenic and contractile myofibroblasts. Upon activation of HSCs both Vitamin A (Vit. A) and lipids are depleted from the HSCs. However little is known about the uptake of Vit A after HSCs activation in liver fibrosis, although one publication mentioned that Vit. A uptake in activated HSCs was as effective as in resting HSCs. (Sato et al). Here we have used Vitamin A-functionalized magnetoliposomes (Vit A-MLs) as MRI contrast agents, which were designed to specifically be taken up by HSCs. They consist of an iron oxide core coated with an anionic lipid bilayer, functionalized with Vit. A residues. Our main objective was to visualize the early onset of liver fibrosis in the rat CCl_4 model with Vit A-MLs as a biomarker for HSC activation and to correlate with the histopathological findings.

Methods: 30 Rats (10 week old male Sprague Dawley male) received up to 8 intraperitoneal injections of CCl_4 (0.8 gm/kg). Within 24 hours after their last CCl_4 injection, all the animals received either functionalized (Vit A-MLs) or anionic non-functionalized MLs; All animals were scanned for MR imaging after last CCl_4 injection and MLs administration. Animals were sacrificed immediately after MR scans and liver tissue processed for histological evaluation on H&E, Van Gieson and iron (Perl's) stains and for macrophages (ED1) and HSC activation (αSMA). Animals were analyzed for their change in the signal intensity post-contrast enhancement. Signal intensities before and after contrast agent administration were compared.

Results: In all rats the liver signal intensities decreased after contrast agent injection, due to the uptake of functionalized/non-functionalized MLs. This decrease in signal intensity (post MLs) was more pronounced in CCl4 dosed rats compared to the vehicle rats, and animals which received functionalized MLs (Vit A-MLs) clearly showed enhanced contrast with lower T2 values compared to non-functionalized MLs. The presence of a 'cobbled stone' (or granular structures) appearance on the liver tissue, post-contrast administration in CCl_4 dosed rats, was considered to be related to the centrilobular-oriented changes caused by CCl_4 injections, and most likely due to the uptake of MLs by the activated centrilobular macrophages (ED1 stain). Histology of the livers, after one to four CCl_4 injections, showed centrilobular congestion with single cell necrosis and chronic inflammation, surrounded by ballooning degeneration and vacuolization of hepatocytes. The first signs of liver fibrosis could be observed after 15 to 18 days and five injections. Changes in the signal intensity were represented in the form of a histogram. From three injections onwards, a shift in signal intensity was noted post-contrast administration. This increase in signal intensity correlated histologically with minimal increase in αSMA staining, indicative for HSC activation/proliferation, after three injections and with slight to moderate HSC activation/proliferation from four injections onwards.

The signal intensity distribution varied with multiple injections, this could be indicative for loss of anatomical structure, probably related to the CCl_4-induced lesions, and at later time points the progression of liver fibrosis.

Conclusion: These preliminary results indicate the possibility to detect the onset and progression of liver fibrosis *in vivo*, using MRI imaging with Vit A functionalized magnetoliposomes. This technique might therefore be valuable in longitudinal studies, to follow the onset, progression and recovery of liver fibrosis in individual animals, in liver disease research and potentially also as a biomarker for clinical use. However due to the limited number of rats used in this study and the confounding inflammatory and degenerative changes caused by the CCl_4 injections, future work is needed to confirm these results.

Reference

Sato et al. Nature Biotechnology Volume 26 number 4, April 2008, p 431–442

Poster Abstracts

P02: Induction of experimental autoimmune encephalomyelitis in cynomolgus monkey with recombinant human myelin oligodendrocyte glycoprotein in incomplete Freund's adjuvant

Anne-Laure Bauchet[1], Krista G. Haanstra[2*], Mireille Doussau[1], Claire-Maëlle Fovet[1], Hélène Touin[1], Laurent Watroba[1], Francois Lachapelle[1], Che Serguera[1#], Bert A. 't Hart[2,3#]*

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[2]Department of Immunobiology, Biomedical Primate Research Centre, Rijswijk, The Netherlands
[3]Department Neuroscience, University Medical Center Groningen, Groningen, The Netherlands
[*]authors contributed equally to the study
[#]authors share senior authorship

The Experimental Autoimmune Encephalomyelitis (EAE) model is based on the immunization of animals with myelin antigens. This model is widely used for preclinical research into the pathogenesis of Multiple Sclerosis (MS) especially in rodents. However, the immature state of the laboratory rodent immune system is considered as a major hurdle in the translation of pathogenic and therapeutic principles from the EAE model to the MS patient. Non-human primates (NHP), who have an immune system harboring T- and B-cell memory against environmental antigens similar to humans, may help to bridge this gap. We report here on the development of a new EAE model in cynomolgus monkeys *(Macaca fascicularis)* induced with recombinant human myelin oligodendrocyte glycoprotein extracellular domain (1–125) (rhMOG) formulated in incomplete Freund's adjuvant (IFA). Clinically evident EAE could be induced in the eight treated cynomolgus monkeys with differences in clinical course and histological lesions. A long evolution (3 animals) was associated with subacute and chronic demyelinating lesions whereas a monophasic course (5 animals) was associated with acute lesions characterized by necrosis, hemorrhage and severeeutrophilic infiltrate. Moreover, the type of evolution correlated with IgM levels. Animals with acute monophasic course had high levels of IgM while those with long evolution displayed low levels of IgM. Thus IgM levels could be an important biomarker to predict outcome and forms of EAE. Histological lesions were mainly associated with neuroaxonal lesions and complement deposition (C9) pointing to a pathogenesis driven by the production of antibodies as often observed in EAE. A model optimization with low production of IgM might give a more appropriate model for testing new therapeutic strategies or study the pathogenesis of human autoimmune leukoencephalopathies.

Poster Abstracts

P03: Reproductive toxicology of soy milk in Swiss Albinos mice

Y. Bouferkas, I. Zeriouh, S. Addou, O. Kheroua, D. Saidi

Laboratory of Physiology of Nutrition and Food Safety (LPNSA) Department of Biology, University of Oran, Algeria

Milk-based soy protein is the main source of phytoestrogens which are similar to the female hormone 17β-estradiol. The objective of this study is to demonstrate the toxic effect of milk on the reproductive system in male mice. Two groups of six male Swiss Albinos mice per group, aged of 4 weeks. The first group received only soy milk and the second group received a standard diet. During the 90 days of the experiment, male mice were weighed every week. At the end of this period, male mice were mated with untreated female mice. Males were then separated and necropsied. Testes, epididymides, and seminal vesicles were processed and examined histologically. An analysis of seminal liquid was performed to determine the motility, morphology and number of spermatozoids. The results showed a significant reduction of the spermatozoids mobility and number and a significant increase of abnormal forms of spermatozoids. The histological changes in the testis consisted in atrophy and alterations of spermatogenesis. The fertility test showed that soy milk reduced the fertility of the treated male mice less fertile when compared to controls. We can conclude that the soy milk has a toxic effect on male reproductive function in mice.

Poster Abstracts

P04: eTOX – Sharing preclinical data & building ontologies to enable better prediction of toxicity

Ms Katharine Briggs

Lhasa Limited

The eTOX project [1] has a number of aims a) to build a database of toxicity data combining public data and legacy reports from participating pharmaceutical companies, b) a common ontology to allow mapping of the terms used across all the different companies and also in public literature to a single preferred term essential for cross-study data analysis & c) a suite of *in-silico* models using this data to better predict the toxicological profiles of new molecular entities in the early stages of drug development.

The current release of the eTOX database contains 2127 preclinical repeat dose studies associated with 246 non-confidential substances and 585 confidential substances with structure related information removed. To evaluate whether there is sufficient data for building *in-silico* models we have analysed the biological space using the common ontology to look at the frequency of key histopathology and clinical chemistry findings.

To analyse how broadly applicable models built on this data would be we have also evaluated the overlap in terms of chemical space with three other datasets – the COSMOS inventory of cosmetic ingredients and related substances [2], the Compendium of Pesticide Common Names compiled by Alan Wood [3] & the Tox21 chemical inventory which covers multiple use-scenarios (pesticides, industrial, food-use, drugs, etc) [4].

In conclusion the numbers of structures associated with key positive findings are considered sufficient for modeling with liver being the main organ affected. Analysis of functional group fragments indicates that the eTOX data occupies different chemical space to the three datasets evaluated. The consortium is currently discussing the conditions under which the data may be made available post project.

References

[1] Inroads to Predict in Vivo Toxicology-An Introduction to the eTOX Project. Briggs K, Cases M, Heard D J, Pastor M, Pognan F, Sanz F, Schwab C H, Steger-hartmann T, Sutter A, Watson D K, Wichard J D. Int. J. Mol. Sci. 2012, 13 (3), p3820–46

[2] Creation of an inventory of known cosmetic ingredients and population with chemical structures at http://www.cosmostox.eu/what/databases/ last accessed 03/05/2013

[3] Compendium of Pesticide Common Names at http://www.alanwood.net/pesticides/ last accessed 03/05/2013

[4] Toxicity Testing in the 21st Century: Implications for Human Health Risk Assessment. Kavlock RJ, Austin CP, Tice RR. Risk Analysis. 2009, 29(4), p485–487

Acknowledgments

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n°115002, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies' in kind contribution. www.imi.europa.eu

Poster Abstracts

P05: Occurrence of amphophilic-vacuolar renal tubule tumors and nephroblastomas in Sprague-Dawley Rats from sub-chronic toxicity studies

Torrie A Crabbs[1], Steve R Frame[2], Victoria A Laast[3], Daniel J Patrick[4], Johnson Thomas[5], Bevin Zimmerman[6], and Jerry F Hardisty[1]

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[2]DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, Delaware, USA
[3]Covance Pharmaceutical R&D (Shanghai) Co., Ltd., Shanghai, China
[4]MPI Research, Mattawan, Michigan, USA
[5]The Dow Chemical Company, Midland, Michigan, USA
[6]WIL Research, Ashland, Ohio, USA

The low background incidence of tumors in rodents from subchronic toxicity studies makes it difficult to assess their relevance, especially when present only in treated animals. This report investigates the occurrence of renal tubule tumors (RTTs), specifically the amphophilic-vacuolar (AV) phenotypic variant, in addition to nephroblastomas, in young Sprague Dawley (SD) rats from sub-chronic toxicity studies. AV tumors are spontaneous, non-treatment-related tumors of familial origin, morphologically distinct from the conventional RTTs induced by exposure to renal carcinogens. They are composed of distinct lobules of large, round to polyhedral cells with vacuolated amphophilic to eosinophilic cytoplasm and prominent nucleoli. Nephroblastomas are rare embryonal tumors that arise from the primordial metanephric blastema and are composed of a variable population of blastematous, epithelial, and stromal cells. Five collaborating laboratories surveyed their subchronic toxicity databases over a ten year span (2002 to 2012). Studies in which one or more of the aforementioned diagnoses had been recorded were included in the dataset. A total of 46 studies contained at least one of the diagnoses: nephroblastoma, n = 10; RTT, n = 13; AV tumor, n = 24 (one study contained a diagnosis of both nephroblastoma and RTT). A total of 10 nephroblastomas were recorded (i. e. one per qualifying study). They were reported in both control (n = 4) and treated (n = 6) groups, and did not exhibit a sexual predilection (male, n = 4; female, n = 6). With regards to the renal tubule neoplasms, the AV tumor variant was reported more commonly than the conventional RTT (n = 45 and 13, respectively), and it was recorded in both experimental (n = 32) and control (n = 13) groups. The AV tumor variant occurred more often in females (n = 34) than in males (n = 11), while the conventional RTTs were recorded more often in males (n = 9) than in females (n = 4). RTTs were recorded only once in each of the 13 studies included in this dataset, while AV tumors often occurred in more than one rat within the same study. AV tumors were documented to occur in rats as young as 7–10 weeks of age. Results from this survey also indicate that AV tumors are being reported more commonly in recent years, with the majority (n = 33) being reported in studies commencing since 2009. While this survey establishes a general profile of occurrence for these tumors, it does not estimate their overall incidence or prevalence. In conclusion, this study reaffirms that AV tumors are spontaneous, non-treatment-related lesions and suggests that they may be more common than conventional RTTs and nephroblastomas in young SD rats. The authors propose that AV tumors be recorded separately from conventional RTTs in order to clearly distinguish the two renal tubule neoplasms and, thus, allow for appropriate interpretation of a compound's potential carcinogenic effect in the kidney.

Poster Abstracts

P06: Centriacinar lung lesions in control rats associated with oral gavage administration

Torrie A. Crabbs[1], Rodney A. Miller[1], David E. Malarkey[2]

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While compound related lung lesions from non-inhalation studies have been described, studies focusing on changes in control animals associated with route of administration are lacking. To determine if there were any differential effects associated with route of administration, lung slides from control male and female F344/N rats were evaluated from the following studies: three chronic gavage studies with corn oil as vehicle control; 2 chronic gavage studies with methylcellulose as vehicle control; one chronic gavage study with water as vehicle control; one chronic water study; one chronic feed study; and two chronic inhalation studies. There were approximately 50 animals/sex/study. Rats from the gavage studies in which corn oil was utilized as the vehicle control exhibited distinct centriacinar lesions that were generally absent in the rats from the other studies. These lesions consisted of a chronic active inflammatory infiltrate that was centered on the bronchiolo-alveolar junction, and was predominately composed of foamy macrophages admixed with lesser numbers of neutrophils and lymphocytes. Bronchiolar metaplasia (bronchiolization) and type II alveolar epithelial hyperplasia were also frequently present. Numerous pale yellow homogenous droplets that stained positively for Sudan Black were commonly present within the alveolar spaces of the rats from these studies. Given that centriacinar lesions are often associated with inhaled intoxicants and that corn oil served as the vehicle control for these studies, the droplets likely represent aspirated corn oil, and the corn oil is likely the culprit for the described lesions. An additional finding was noted in several of the males ($n = 15$) from one of the corn oil gavage studies: multifocal random aggregates of macrophages that, in some instances, formed discreet granulomas. These lesions were often noted grossly as small white disseminated foci and were commonly accompanied by chronic perivascular inflammation. There were no additional findings in any of the remaining studies, including the oral gavage studies in which water or methylcellulose was used as the vehicle control, associated with route of administration; however, spontaneous alveolar histiocytosis was commonly present, irrespective of administration route. While it is possible that respiratory tract lesions in non-inhalation studies represent a systemic effect of the test compound, this study demonstrates that additional mechanisms for exposure must be considered, especially in the case of oral gavage studies where technical gavage errors, gavage-related reflux, spontaneous reflux, and accidental aspiration can all occur. Given the lack of significant information on lung lesions in control animals, these findings will serve as a useful addition to the literature and will add insight to the possible pathogeneses of lung lesions in animals from non-inhalation studies.

Poster Abstracts

P07: The effects of Protein Kinase C inhibitors on steroid hormones: a 2-week rat oral (gavage) investigative study focusing on pathology and hormone analysis in plasma and tissues

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The Protein Kinase C (PKC) family is a family of serine/threonine kinases with various classical and novel isoforms. Some of these isoforms are expressed in T and B cells and have a key role in T-lymphocyte activation, downstream of the T-cell receptor and CD28 co-receptor signaling, therefore being used as immunosuppressive agents. PKC could also have a potential role as a receptor and/or transducer of the non-genomic effects of steroid hormones which regulate a wide variety of cellular responses (regulation of ion transport and cell proliferation/migration/differentiation, and death) (Alzamora and Harvey, 2008). The inhibition of PKC could alter steroid hormone actions and regulation leading to disturbances in multiple organ systems, particularly the reproductive system.

To investigate the potential effects of PKC inhibitors on steroid hormones and its consequences, a 2-week oral mechanistic study in rats was conducted with a PKC inhibitor at two doses, focusing on pathology and hormone analysis in plasma and tissues. In males, tubuloalveolar pattern (feminization) in the mammary glands was observed microscopically and hormone analysis showed decreased androstenedione and testosterone concentrations in plasma, adrenals and testes. In females, atretic follicles and corpora luteal degeneration in ovaries were present microscopically, accompanied with disturbed estrus cycle, as evident in the vagina histology and vaginal smear cytology. Hormone analysis revealed decreased estrogens and its precursor androstenedione in the ovaries. Progesterone was increased in ovarian and adrenal tissues, whereas it was decreased in plasma. In both sexes, Luteinizing Hormone (LH) was decreased in blood. LH decrease was considered responsible for the reduction of androgens, estrogens and plasma progesterone which in turn led to the observed disturbances in estrous cycle. However, as progesterone levels were elevated in ovaries, it was thought that PKC inhibitor might have an additional direct effect in the corpora lutea which is independent of the LH reduction. In both sexes, aldosterone was elevated in plasma and adrenal glands associated with increased cortical vacuolation microscopically.

Lymphoid depletion of various lymphoid organs (thymus, lymph nodes and spleen) was seen in both sexes at both doses and consistent with known immunosuppressive effects of PKC inhibitors. Absent/decreased germinal centers of lymph nodes were also supportive of the inhibitory properties of PKC inhibitors on lymphocyte activation and proliferation (Matz et al, 2011).

In summary, the results of this study support possible PKC inhibitors induced hormonal imbalances in the rat, likely due to a disturbance of the hypothalamus-pituitary-gonad axis and/or direct effect on the ovaries and adrenals. The results also confirm the expected immunosuppressive effect of PKC inhibitors.

References

Alzamora and Harvey (2008). Direct binding and activation of protein kinase C isoforms by steroid hormones. Steroids 73 (9–10), 885–888.
Matz et al (2011). Evaluation of the novel protein kinase C inhibitors otrastaurin as immunosuppressive therapy after renal transplantation. Expert Opin Drug Metab Toxicol, 7 (1): 103–113.

Poster Abstracts

Po8: Spontaneous complex odontoma arising from a molar tooth in a Syrian hamster

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In rodents, odontogenic tumors almost exclusively arise from the incisors due to their continuous and lifelong growth. In contrast, tumors deriving from molars are extremely rare because the crowned and rooted molars have a short developmental time with early degeneration of the epithelial root sheath soon after formation of the root dentin.

The complex odontoma was observed as incidental finding adjacent to the 2nd left maxillary molar in a 105-week-old female Syrian hamster (strain: Han: AURA) which served as untreated control animal in a 24-month carcinogenicity study.

Histologically, the tumor appeared as small nodular mass, surrounding the molar tooth and partly extending into the nasopharynx. Parts of the molar and adjacent alveolar bone were distorted and resorbed. Plant-derived foreign material was inspissated multifocally in the tumor periphery and associated with a purulent inflammation. The tumor tissue showed a characteristic poor morphodifferentiation with little resemblance to normal tooth. Irregularly-arranged strands and islands of dental hard tissues such as enamel, dentin and cementum were intermingled with ameloblastic epithelium, odontoblasts, cementoblasts and dental pulp-like mesenchymal cells. The ameloblastic cells were well-differentiated and associated with dental hard tissues resembling normal odontogenesis. There was also some proliferation of gingival squamous epithelium around the tooth margin.

Pathogenetically, the present tumor is considered to be derived from proliferated so-called 'epithelial rests of Malassez' which remain in the periodontal ligament after eruption of the tooth. Proliferation of these cells was probably triggered by foreign-body-related irritation and inflammation of the periodontium.

Poster Abstracts

P09: Semi-automated quantitative image analysis for the glomerular markers desmin and WT1 in a Dahl salt sensitive rat model after unilateral nephrectomy – effect of Lisinopril and the combination with hydrochlorothiazide (HCTZ) on kidney function and morphology

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Introduction and Aims:

Dahl salt sensitive rats develop hypertension and renal injury under high salt diet. The aim of the present study was to establish quantitative image analysis for the glomerular immunohistochemical markers desmin and Wilm's tumor protein (WT1) in order to investigate the effect of unilateral nephrectomy (UNx) in Dahl salt sensitive rats pre-fed for 2 weeks with 8 % NaCl diet on the progression of kidney disease. In addition, the treatment effects on kidney function and morphology of Lisinopril and the combination with the diuretic hydrochlorothiazide (HCTZ) was assessed.

Methods:

Kidney function was assessed by GFR (plasma clearance kinetics of fluorescein isothiocyanate inulin), creatinine (CREA) clearance and urinary albumin. Kidney morphology was evaluated from H&E and PAS stained formalin-fixed and paraffin-embedded (FFPE) tissue sections. Immunohistochemistry on FFPE tissue sections for desmin and WT1 and image analysis was performed with the Ventana Discovery XT immunostainer and the Definiens Tissue Studio software.

Results:

UNx versus sham rats

- CREA clearance and GFR was decreased in the UNx rats when compared to sham rats.
- UNx rats had increased albuminuria.
- Both degenerative glomerular and tubulo-interstitial histopathological lesions were increased in UNx rats.
- Desmin in the glomeruli as a marker for podocyte damage was significantly increased and the number of WT1 positive podocytes was significantly decreased after UNx when compared to sham rats.

Lisinopril (100mg/kg/d) versus Lisinopril (100mg/kg/d) with HCTZ (15mg/kg/d)

- 4-week treatment of UNx rats with Lisinopril alone neither improved CREA clearance nor GFR, whereas the Lisinopril/HCTZ combination preserved both parameters.
- Lisinopril alone did not lower albuminuria, whereas the Lisinopril/HCTZ combination decreased albuminuria.
- Lisinopril alone was not able to preserve normal kidney morphology, whereas the combination of Lisinopril/HCTZ was able to prevent both glomerular and tubulo-interstitial histopathological lesions.
- Podocyte damage (desmin) was significantly lower and the number of podocytes (WT1) was significantly higher after combination of Lisinopril/HCTZ when compared to Lisinopril alone.

Conclusions:

This experiment demonstrates that UNx exacerbates renal disease progression in Dahl salt sensitive rats on a high salt diet. In addition to kidney function, image analysis results for the glomerular markers desmin and WT1 provided confirmatory data for both disease progression and prevention of tissue damage. ACE inhibitor Lisinopril alone was not able to protect kidneys from further damage under this experimental setting. Combining Lisinopril with the diuretic HCTZ was able to prevent worsening of kidney function and morphology. This animal model might offer the possibility to study effects on top of ACE inhibitor treatment.

Poster Abstracts

P10: Acute oral, subchronic oral and dermal toxicity studies in Sprague-Dawley rats treated with agrochemical fungicide bellum 33.4%

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Acute oral, subchronic oral and dermal toxicity studies of BELLUM 33.4% WG (PYRACLOSTROBIN AND BOSCALID 6.7 AND 26.7%), an agrochemical fungicide, in adult Sprague-Dawley rats, were conducted. Eight rats were used in each of the treatments and the untreated control group. Initial and final body weights (BW) of rats and organ weights (OW) were recorded. Tissues and blood samples were also collected and examined. Analysis of the results showed that the acute oral toxicity (LD50 in 48 hours) is greater than 1700 mg/kg. The subchronic oral toxicity studies (14 days with 17 mg/kg) revealed no difference in body weight gain with an increase BW in both groups of 19% in the treated vs 24% in control. Packed Cell Volume (PCV), Hemoglobin (Hgb) percentage and absolute number of lymphocytes (2993 vs 2803), total proteins, ALT and AST were increased in the treated group compared to the control. There was a slight decrease in the organ weight of the liver, kidneys, heart, lungs and spleen in the treated group. In addition, the results of the subchronic dermal toxicity study (14 days with 17 mg/kg, dermal application) indicated similar changes. There was also an increase in RBC, PCV, Hgb, total proteins, and decrease in WBC, ALT and AST in the treated group compared to the control group. These changes were not significant. There was only mild hydropic degeneration associated with dilatation of the sinusoids in the liver and mild tubular vacuolation in the proximal tubules of the kidney in the orally treated groups, and mild shrinkage in some of the glomerular tufts of the kidney in the dermal treated group. In addition, no significant histopathological alterations were seen in the skin or any other organs examined as a result of the 14 days dermal or oral administration. These three studies collectively suggested that BELLUM 33.4% WG is slightly toxic, and has to be classified in category 4 in the Global Harmonized System (GHS). Tables with means of values and comparison of the histopathological profile will be presented and discussed in the poster presentation.

Poster Abstracts

P11: Supporting the need for International Harmonization of safety assessment for Food Flavoring Substances

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Flavoring substances (FS) are food ingredients that are used to give olfactory impact (taste and/or smell) to food. In the United States (USA) and European Union (EU) regulatory guidelines for use of FS in or on food differ from rules applicable to other groups of food additives. FS can be approved for use using the FEMA (Flavor and Extract Manufacturers Association) GRAS (Generally Recognized as Safe) process in the USA and EFSA (European Food Safety Authority) guidelines in Europe. In the USA and EU, regulatory approval for permitting use of a FS includes evaluation of relevant data, which in some cases includes genotoxicity and toxicity testing data on the FS itself, but also uses in silico data (intended levels in specific foods, purity & specifications), QSAR (Quantitative Structure-Activity Relationships, metabolic and pharmacokinetic characteristics), use of Read-Across Strategies, and application of TTC (Thresholds of Toxicologic Concern) with ultimate determination of safety by qualified expert scientists. This approach is pragmatic in light of traditional long-term use of thousands of FS typically in parts per million (ppm) to parts per trillion (ppt) levels in foods and beverages. In contrast to the USA and EU approach, safety assessment of FS in Japan requires in vivo and in vitro genotoxicity data as well as repeat-dose 90-day animal toxicity data for every FS. Given the increasing global interest in consumption of ethnic foods, the fact that hundreds of FS are in use in Japan and potentially available for export, and the nature of contemporary international commerce, we strongly encourage the need for international harmonization of the safety assessment of FS for use in foods. Here we propose a decision-tree approach for international harmonization and standardization of safety assessment of FS for foods and beverages.

Poster Abstracts

P12: Local effects of various microelectrodes implantation in rabbit muscles

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Microelectrodes are essential for various prosthesis. Because electrical signal is transmitted to the microelectrode embedded in the patient's muscle, which stimulates electrically the remaining muscles. The purpose of this study was to evaluate the biocompatibility of various polymer-based microelectrodes (PBMs) after implantation in rabbit muscle tissues following a standardized method. Three types of PBMs were examined: silicone-based platinum (SP), polyimide-based gold (PG), and liquid crystal polymer-based gold (LCPG) microelectrodes. All experimental procedures followed the International Organization for Standardization (ISO) 10993-6:2007(E). Six female rabbits were used for this study. The PBMs were implanted into the left paravertebral muscle of the dorsal region of the rabbits for 12 weeks, each type being implanted into two rabbits. Control article (high density polyethylene, HDPE) was implanted in the equivalent site on the right side of each rabbit. No changes in the clinical signs, mortality, body weight, and gross findings related to the PBMs were noted. At the histopathological examination, mild inflammatory reaction with foreign body reaction and fatty ingrowth were increased in all three test groups compared to control group, but severe necrosis and infection were not identified in all test groups. As histopathological scoring according to ISO 10993-6, the irritant ranking conclusion of test articles was evaluated as 16.92 (PG), 7.01 (SP) and 11.50 (LCPG). Therefore, SP microelectrode is considered to be the less irritant materials and is biocompatible enough to be a candidate for various prosthesis.

Poster Abstracts

P13: Possible contribution of cell proliferation to gene mutation following exposure to the hepatocarcinogen estragole

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DNA modifications are considered to be a trigger for somatic mutations. However, there are various factors such as DNA repair and cell proliferation contributing to the process from DNA modification to gene mutations. Estragole (ES), a natural constituent of several herbs and spices, is carcinogenic in the rat liver. As determined by LC-MS/MS analysis, ES forms several DNA adducts, including ES-3'-N2-dG, ES-3'-C8-dG and ES-3'-N6-dA in the rat liver. We also found that these DNA adducts were detected to some extent in the livers of rats treated with ES even at the low dose with the lack of in vivo mutagenicity. To clarify essential factors contributing to the genotoxicity of ES, we examined quantification of the ES-specific DNA adducts, reporter gene mutation assay and cell proliferation in the livers of F344 gpt delta rats given ES at doses of 0, 3, 30 or 300 mg/kg/day by gavage during 4 weeks. The relative liver weights were significantly increased and characteristic histopathological findings such as mitosis, single cell necrosis in hepatocytes and oval cell proliferation were found at the highest dose. However, serum AST and ALT levels were not remarkably increased (about 1.4-fold and 1.7-fold, respectively) in spite of the values being statistically significant. Three types of ES-specific DNA adducts were detected at all doses and in a dose-dependent manner. Nevertheless, the gpt and Spi- mutant frequencies were significantly increased only in the high-dose group. These results may imply that the mutagenicity of ES is not only dependent on the amount of modified bases. In addition, PCNA-positive cells, expression of cell cycle-related genes, such as Cyclin A2, B2, and E1, and increased phosphorylation of the ERK protein were observed only in the high-dose group. These findings suggest that cell proliferation may be a prerequisite for the progression from DNA modifications to gene mutations. Given that ERK phosphorylation regulates the progression of the cell cycle, clarification of the upstream factors that phosphorylating ERK may be warranted to understand the mode of action underlying ES-induced cell proliferation.

Poster Abstracts

P14: ZSF1 rats and kidney: Exacerbation of nephropathy by unilateral nephrectomy and efficacy of Lisinopril in ameliorating the lesions

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INTRODUCTION AND AIMS:

Diabetic nephropathy (DN) in humans is a complex pathology leading to end stage renal disease (ESRD). Development of treatments that prevent ESRD requires translatable models and evaluation of biomarkers. Unilateral nephrectomy (UNx) is known to reduce glomerular filtration rate (GFR) and induce proteinuria in animal models and human. Therefore the aim of the study was to explore UNx as a mean of exacerbating nephropathy in ZSF1 rats which are a model for DN. In addition, the effect of the hypotensive and kidney protective drug Lisinopril (ACE inhibitor) as therapeutic on the development of nephropathy was investigated.

METHODS:

Hypertensive and metabolic syndrome male ZSF1 rats were submitted to unilateral nephrectomy (UNx) of the left kidney at 12 weeks of age; sham operated ZSF1 rats served as controls.

Clinical Analyzer, ELISA and Luminex® technology were used to quantify urine and plasma biomarkers (BMs). Impact of UNx on kidney structure was determined by histology (H&E and PAS) of formalin-fixed and paraffin-embedded tissue sections.

Sensitivity of the UNx-ZSF1 rat model to intervention with ACEi Lisinopril (at 30mg/kg/day; as food admix, from 3rd week after UNx) was evaluated during chronic treatment (for 12 weeks) on kidney BMs, function and structure; vehicle treated UNx-ZSF1 rats served as controls.

RESULTS:

Compared to age-matched sham operated ZSF1 rats, UNx increased right kidney size and weight (absolute and relative to body weight), reduced urine volume, Creatinine clearance and deteriorated several plasma parameters (e.g. BUN and KIM-1) and urine BMs (e.g. Albumin, Cystatin-C and GDF-15), and increased severity of kidney changes such as glomerulosclerosis, glomerular vacuolation, thickening of basement membrane, tubulointerstitial inflammation and fibrosis, tubular dilation and proteinaceous casts. Lisinopril significantly reduced kidney size, improved glomerular filtration rate, normalized albuminuria, reduced KIM-1, β2microglobulin, Cystatin-C and GDF-15 excretion and reduced incidence and severity of renal microscopic changes.

CONCLUSIONS:

The data confirms that the ZSF1 rat model develops DN with morphological similarities to human DN. UNx worsened DN progression in male ZSF1 rats as compared to sham operated ZSF1 rats. Chronic treatment with Lisinopril significantly reduced renal injury (structure, functions and improvement of BMs). The UNx_ZSF1 rat model could be used for exploring further mechanism of DN and demonstrating protection of kidney function by new drugs.

Poster Abstracts

P15: Strain differences in neurohistopathology and morphometry outcome in methylazoxymethanol treated rats

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Introduction:

Neurohistopathology and morphometric assessment are important tools to evaluate neurodevelopmental effects in rats exposed to potentially toxic compounds. For these neurotoxicity testing studies Sprague Dawley (SD) rats are most commonly used, especially in the US. In Europe the Wistar-Han (WH) rat is the more commonly used rat strain in routine toxicity testing. There is a lack of information on the differences in response between these two rat strains in a developmental neurotoxicity testing (DNT) environment. In the present study we investigated the differences in DNT pathology endpoints between these two rat strains and their acceptability for DNT studies, using the positive control agent methylazoxymethanol acetate (MAM).

Material & Methods:

Female WH and SD rats were bred with male rats of the same strain. On gestation day 15, 20 mg/kg MAM was injected intraperitoneally in pregnant females. The control group received 0.9% NaCl. The *in utero* exposed pups (10 animals/sex/treatment) were perfusion-fixed using paraformaldehyde and necropsied on postnatal days (PND) 21 or 70–73. Brain weights (and terminal body weight) were recorded from all animals. Central nervous (CNS) tissues were collected for animals at PND 21 and CNS and peripheral nervous (PNS) tissues for animals at PND 70–73. Slides were prepared of 10 animals/sex/treatment for both necropsy days. The PNS tissues were embedded in plastic and 1 µm slides were prepared. The CNS tissues from both necropsy periods were embedded in paraffin and sections of 2–3 µm were prepared. Slides were routinely stained using H&E. Additionally, CNS tissues were stained using Luxol fast blue/Cresyl violet to facilitate morphometry. Histopathology and morphometric analysis was performed.

Results of morphometry:

There was a marked decrease in the height of the cortical hemispheres and the vertical and radial cortical thickness in the MAM treated WH and SD rats at both PND 21 and 70–73. This effect was more pronounced in the SD rats. In addition, in the SD rats, a decrease in the length of the ventral limb of the dentate hilus was measured at both PND 21 and 70–73 and a reduced vertical height of the dentate hilus at PND 70–73.

Results of histopathology:

PND 21; In WH and SD rats abnormal neuronal lamination in the cerebral cortex was observed in both sexes, as well as malaligned CA1 and CA2 pyramidal cells in the hippocampus (characterized by focal hypocellularity and heterotopia) in male WH rats and in male and female SD rats. In addition, in SD rats only, there was hypoplasia of the corpus callosum in the brain of both sexes.

PND 70–73; There was hypoplasia of the corpus callosum in the brain of female WH and male and female SD rats and there was abnormal neuronal lamination in the cerebral cortex and malaligned CA1, CA2 and CA3 pyramidal cells in the hippocampus of male and female WH and SD rats.

Conclusion:

SD rats were much more affected than WH rats in the incidence, type and severity of the effects seen in the morphometric analysis and histopathology. For histopathology this particularly involved the corpus callosum hypoplasia and CA1 and CA2 pyramidal cell malalignment in the hippocampus.

WH and SD rats are both acceptable for standard use in the DNT study since they have mostly similar endpoints. Attention is warranted when utilizing agents like MAM. Moreover, this study indicates that the potential differences between different strains should always be taken into account.

Poster Abstracts

P16: Evaluating the eye irritation potential in BCOP assay by a semiquantitative histopathological method

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Introduction:

The BCOP (bovine corneal opacity and permeability) test is an *in vitro* method for the evaluation of potential severe eye irritation/corrosion of test substances. In addition to the standard measurements (opacity and permeability) in the BCOP as laid out in OECD test guideline 437, histopathology of the treated corneas has been suggested as valuable parameter to improve the prediction of the eye irritant potential. However, until now, no consistent criteria exist for the histopathological diagnosis of bovine corneal irritation in the BCOP assay. We have developed a histopathological method that reflects the *in vivo* irritant potency of test materials. Methods: *Ex vivo* bovine corneas were exposed to 31 industrial chemical substances and 50 agrochemical formulations in the BCOP test (triplicates for each test substance were used). The corneas were fixed in 10 % neutral-buffered formalin, processed histotechnically and stained with hematoxylin and eosin for light microscopical examination. In a first step, the type and depth of injury (DOI) in the epithelium and stroma were evaluated. In the epithelium, a standard semi-quantitative grading system was used, whereas in the stroma the type and DOI was recorded in percentage of affected tissue extending from the epithelial basement membrane throughout the stroma. In a second step, epithelial and stromal evaluations were combined according to established cut-off criteria resulting in a final histopathological score of irritation (HSI) ranging from I (minimal) to IV (severe). Results: Test substance specific histomorphological patterns were observed and were reproducible. The HSI was predictive for the *in vivo* Draize test classification according to GHS (Globally Harmonized System of Classification and Labeling). Histopathological evaluation reduced the underprediction rate for severe irritating agrochemical formulations but not for severe irritating industrial chemicals. Conclusion: This study provides evidence, that corneal histology may offer valuable additional information to the standard endpoints assessed in the BCOP test.

Poster Abstracts

P17: Modification of combined administration of CYP inducers in rat liver tumor promoting activity

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Introduction:

Among the nongenotoxic liver carcinogens, some cytochrome P450 (CYP) inducers have liver tumor-promoting activity in rats involving oxidative DNA damage through generation of microsomal reactive oxygen species (ROS). We hypothesized that simultaneous exposures to these CYP inducers can enhance their carcinogenic or tumor-promoting effects. In this study, we performed a rat two-stage liver carcinogenesis bioassay to examine the modification of tumor-promoting effect by co-administration of CYP2B inducers, phenobarbital (PB) and orphenadrine (ORPH), or PB and piperonyl butoxide (PBO, a CYP1A/2B inducer).

Materials and Methods:

Male rats were given a single intraperitoneal injection of *N*-diethylnitrosamine (DEN) and were treated 2 weeks after the DEN initiation with (1) PB (60 or 120 ppm) in drinking water, ORPH (750 or 1500 ppm) in diet or 60 ppm PB + 750 ppm ORPH; (2) PB (60 or 120 ppm) in drinking water, PBO (1250 or 2500 ppm) in diet or 60 ppm PB + 1250 ppm PBO for 6 weeks. Two-thirds partial hepatectomy was performed one week after the start of CYP-inducer treatment.

Results:

The liver samples showed centrilobular hepatocyte hypertrophy with eosinophilic cytoplasm in the PB- and/or PBO-treated groups and diffuse vacuolar degeneration in the PB-treated groups. Eosinophilic hepatocellular altered foci were also observed in the High PB, High PBO and PB+PBO groups. (1) The number of glutathione S-transferase placental form (GST-P)-positive foci and the mRNA expression level of *Cyp2b1/2* significantly increased in the PB+ORPH group compared with the average of the High PB and High ORPH groups or the sum of the net values of the Low PB and Low ORPH groups. (2) The area of GST-P-positive foci and the mRNA expression level of *Cyp1a1* in the PB+PBO group were significantly lower than the average of the High PB and High PBO groups.

Discussion:

These results suggest that the combined administration of CYP2B inducers enhances the liver tumor-promoting activity, in contrast, the co-administration of CYP2B inducer and CYP1A/2B inducer inhibits the activity in rats.

Poster Abstracts

P18: Nigella sativa oil protects against reproductive toxicity of acetamiprid insecticide in male rats

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Purpose:

Acetamiprid (ACMP) is one of the main neonicotinoides used as insecticide on a large scale in agricultural activities. It has been implicated in several health problems in mammals through its capacity to generate oxidative stress in several organs. Until now, little is known about its adverse effects on the reproductive function. Hence, the present study was designed to investigate the adverse reproductive effects of acetamiprid, besides the possible protective role of Nigella sativa oil (NSO), as a potential antioxidant agent.

Methods:

Thirty two male Wistar rats were allocated into four equal groups of eight, control, acetamiprid (ACMP, 27 mg/kg), Nigella sativa oil (NSO, 0.5 ml/kg) and in combination (ACMP + NSO). The experimental animals were dosed by gavage (5 day on a week) for 45 consecutive days. Body weight gain, reproductive organs weights, sperm characteristics, testosterone and thiobarbutiric acid- reactive substances (TBARS) levels and testicular histopathological changes were investigated.

Results:

The obtained results showed that acetamiprid induced deleterious effects on the general health state and the semen quality associated with decrease in body weight gain, relative weights of reproductive organs (testis, epididymis and seminal vesicle), spermatids number, sperm count, sperm motility and testosterone levels and with increase abnormal and dead sperm and TBARS level. Histopathological examination of acetamiprid testis group revealed tubular atrophy, disorganization and degeneration of the seminal epithelium in some seminiferous tubules associated with spermatogenesis perturbation, no to low sperm content and presence of slough cells in the lumens, edema and hemorrhage. In the meantime it was shown that the co-administration of Nigella sativa oil along with acetamiprid can efficiently reverse and/or modulate all acetamiprid-induced reproductive adverse effects. This protective role may be due to its antioxidant properties and ability to reduce TBARS levels as shown in this work.

Poster Abstracts

P19: Intraperitoneal administration of high doses of polyethylene glycol (PEG) causes hepatic subcapsular necrosis and low-grade peritonitis with a rise in hepatic biomarkers

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Polyethylene glycols (PEGs) are high molecular weight oligomers or polymers of ethylene oxide, commonly employed as excipients in preclinical studies and in vitro experiments to dissolve poorly hydrosoluble drugs. Their use is generally considered safe in both animals and humans; however, limited data are available concerning the safety of PEGs when administered parenterally. As part of a larger study on drug-induced liver injury, CD1 and C57BL6 mice were administered a single intraperitoneal (ip) injection of furosemide (FS, 400mg/kg) in PEG-400 (4 ml/kg) or the vehicle alone (PEG-400, 4 ml/kg). Using light and electron microscopy and serum markers of liver injury as well as an in vitro approach, we were able to demonstrate that PEG-400 in a high dose has an irritant effect on serosal surfaces and causes subcapsular hepatocellular necrosis in mice when administered intraperitoneally, along with a rise in ALT and miRNA levels, and has a cytotoxic effect on hepatocytes. Accordingly, levels of serum biomarkers of liver injury need to be carefully interpreted in studies where PEG is administered intraperitoneally and always in association with the results of the histological assessment.

Poster Abstracts

P20: An overview of the modified National Toxicology Program approach to neuropathology

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The National Toxicology Program has recently adopted a modified approach in the neuropathology evaluation of the rodent brains in routine toxicology and carcinogenicity studies. The modified approach includes the evaluation of the traditional three sections and an additional four coronal sections resulting in a total of seven coronal sections. These include a section through the mid-olfactory bulb; a section through the fronto-parietal cortex including basal ganglia; a section through the mid-parietal cortex and thalamus; a section through the mid-brain with substantia nigra; a section through the inferior colliculi; a section through the mid-cerebellum including cranial nerve VIII; and a section through the caudal medulla through the area postrema. Incorporation of this modified approach allows evaluation of at least 50 neuroanatomic subsites. Selective inclusion of these subsites are based on unique vulnerability of specific subsites to toxicants such as the olfactory bulb, inferior colliculus and area postrema, and those relevant to neurodegenerative diseases such as substantia nigra in Parkinson's and Huntington's diseases. This poster displays the landmarks and procedural details for brain cutting to obtain the seven sections in the rat and mouse. For toxicologic pathologists, an understanding of the organization and functional neuroanatomy of the normal brain is the basis for any assessment of pathological changes induced by toxicants. With respect to this, an overview of the neuroanatomic subsites noted at each level in the context of functional neuroanatomy is included.

Poster Abstracts

P21: Surface modification and different crystallinity of titanium dioxide nanoparticles cause similar histopathological lesions following inhalation in Wistar rats

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Introduction:

Nanomaterials and nanoparticles are gaining more and more importance in science, technology, and medicine. However, increasing usage and distribution of these materials may cause a health risk. Additionally, surface modifications of nanoparticles and different crystallinity might influence their toxicological potency.

Methods:

To investigate whether differences in surface and crystallinity of nanomaterial affect deposition and histopathological lesions, three different TiO_2 nanoparticles, each with three increasing doses, were used in a 28-day nose-only inhalation study in Wistar rats (Crl: WI(Han)). These nanoparticles were NM-103 (hydrophobic, rutile), NM-104 (hydrophilic, rutile), and NM-105 (hydrophilic, 80 % anatase/20 % rutile) in a dosage of 3, 12 or 48 mg/m^3. Following a recovery period of 3, 45 or 94 days after the end of the 28-day inhalation study, the animals were sacrificed and the respiratory tract including nasal cavity, larynx, trachea, lung and lung-associated lymph nodes (LALN) were processed for histopathological examination.

Results:

Particle-laden macrophages were seen dose-dependently predominantly within the alveoli in the lung and at a lower amount in the LALN, BALT, lung interstitium, and rarely subepithelially in the nasal cavity, larynx, and trachea. Interstitial accumulation of particle-laden macrophages was accompanied dose-dependently by very slight interstitial fibrosis and interstitial mononuclear cell infiltration increasing partly to slight at recovery day 94. In the mid and high dose groups of all three investigated nanoparticles very slight to slight intra-alveolar granulocytic cell infiltration and remnants of degenerated macrophages were visible. Furthermore, particles were occasionally found intracellular within the lympho-epithelium covering the NALT and BALT. In summary, all three treatment groups showed similar dose-dependent lesions and a similar deposition and distribution pattern of particles in the respiratory tract.

Discussion:

Though a different surface modification and crystallinity might have suggested no obvious differences between the three different treatment groups in terms of degree and character of induced lesions were observable by histopathology.

Poster Abstracts

P22: Detecting and quantifying liver pathologies using compact, high-resolution 3D MRI-based Histology

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Magnetic Resonance Imaging (MRI) is widely used in pre-clinical research and provides a powerful method for *in vivo* assessment of phenotypes in small animal models of disease. MR Histology (MRH) (Johnson et al) of fixed tissue specimens is gaining recognition as a technique to provide complimentary information to conventional histology, as numerous digital slices from any plane can be acquired in the intact sample. Moreover, non-destructive quantification of 3D structures allows specimens to be imaged and then sectioned for conventional histology. With the advent of new compact MR systems that are designed to operate in most conventional labs without the cost, complexity and infrastructure needs of conventional MRI systems, the possibility of MRI- based histology becoming wide-spread is now viable.

The purpose of this study was to investigate the capabilities of a new compact, high-performance MRI platform (M2, Aspect Imaging) in detecting and quantifying liver pathologies manifested in mice with homozygous disruption of the mdr2 P-glycoprotein gene. These mice are unable to secrete phospholipids into bile causing chronic hepatic inflammation manifested shortly after birth leading to the development of pre-neoplastic lesions which progress thereafter to hepatocellular carcinomas (HCC).

Mdr2 (-/-) mice were anesthetized and placed on a compact MRI scanner equipped with a specially designed heated mouse bed and a 35 mm mouse body radio frequency (rf) coil. Physiological parameters were monitored throughout the scan in order to assure animal wellbeing. Focal liver lesions were detected in vivo in all 16-month old mice (n = 6) using the M2 compact MRI scanner. Livers were then extracted and fixed in formalin and high-resolution ex-vivo MRH of the extracted organs was performed using a 20mm rf coil on the same compact MRI platform. Different types of focal lesions were detected in the MRH scans, later characterized by H&E histology as focal fatty changes (n = 3) and cystic HCC (n = 3). 3D MRH of the fixed livers was helpful in detecting and quantifying as many as 15 lesions in one single liver.

We have demonstrated the utility of compact, high-performance MRI and 3D MR-based histology as valuable tools to complement conventional toxicological studies. Conventional toxicology studies suffer from the limitations of histology based results in which significant amount of time is required, limiting the investigation of target organs to a small number of 2D slices. MRH allows for rapid acquisition of 3D data of the entire target organ, leading to a more comprehensive assessment of the toxicological effects and its extent.

While *in vivo* MRI can provide an invaluable tool for detection of onset and progression of disease by non-invasively imaging the same animals over time, non destructive *ex vivo* MRH provides high throughput and high-resolution 3D digital data sets of intact organs, with exquisite morphological and quantitative information. With a high degree of correlation to conventional H&E, 3D MR-based histology can provide both additional insights into disease pathology as well as directing conventional histology to ensure key targets are fully assessed, considered and calculated in toxicological work-ups.

Poster Abstracts

P23: The relevance of the guinea pig as species for repeated toxicity studies with human monoclonal antibodies

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According to "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (ICH S6)", due to the species specificity of many biotechnology-derived pharmaceuticals, it is important to select the relevant animal species for toxicity testing. If guinea pigs are expected to have a pharmacological activity to biotechnology-derived pharmaceuticals based on the expression of the receptor or epitope information, guinea pigs could be one of the relevant animal species for toxicity testing. On the other hand, guinea pigs are known to be highly susceptible to anaphylaxis following exposure to foreign proteins. The objective of this study is to confirm whether a guinea pig is adequate as an animal species for toxicity study with human or humanized monoclonal antibody drugs or not. In this study, human Immunoglobulins (Glovenin-I, hereinafter referred to as IgG) at doses of 0 (vehicle), 0.1, 1, 10 and 100 mg/kg were administered intravenously once weekly to male guinea pigs (n = 5/dose group).

In the 10 mg/kg group, death was observed in three animals, and moribundity in the remaining two animals. In the 1 mg/kg group, moribundity was observed in two animals. These seven animals died or became moribund 1–2 minutes after the 3rd dosing on Day 15. Surviving animals were not dosed more than 3 times and were terminal sacrificed on Day 22 for animal welfare reason. In these animals, the main test article-related changes were abnormal clinical signs such as nose scratching, convulsion, dyspnea, restless and lateral position. These clinical signs strongly suggested hypersensitive response. Decreases in platelet and increases in lymphocyte were also observed in these animals. The surviving animals in 0.1, 1 and 100 mg/kg groups showed the above-mentioned clinical signs after the 2nd or 3rd dosing, and tremor after the 3rd dosing in the 1 mg/kg group and reddening of pinna on the following day of the 2nd or 3rd dosing in the 1 and 100 mg/kg groups were also noted. Therefore, surviving animals were not dosed on Day 21 and were sacrificed on Day 22.

To evaluate immunization status, some animals were devoted to a skin test after the 3rd dosing. In the 1 and 100 mg/kg treated animal, intracutaneously injected human IgG caused a very slight blue patch/zone subcutaneously in skin test due to 0.5 % Evan's Blue solution, suggesting hypersensitivity reaction with increased permeability of vessels within human IgG injected site.

At histopathology examination, some of the dead or moribund animals showed perivascular edema in lung and submucosal edema in esophagus. These findings strongly indicated that hypersensitive responses to human IgG occurred when human IgGs were intravenously administered to guinea pigs.

Based on these results, guinea pig would not be a suitable animal species for repeated intravenous dose toxicity study of human antibody drugs.

Poster Abstracts

P24: Modifying effects of sulforaphane on diethylnitrosamine or furan induced liver carcinogenesis in rat

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NRF2 (nuclear factor erythroid 2-related factor 2) is a transcriptional factor that regulates expression of a number of phase II detoxification and antioxidant enzymes in response to cellular events such as electrophilic and oxidative stress. Sulforaphane (SFN), an activator of NRF2, suppresses chemical induction of mammary and colonic tumors in rats. In contrast, somatic mutations in *NRF2* resulting in constitutive activation of the NRF pathway have been reported in several human cancers, suggesting that NRF2 also contributes to tumor growth. Activation of the NRF2 pathway results in two opposing effects on the process of carcinogenesis. However, the impacts of NRF2 activation and the steady-state situation in tumor cells, especially in preneoplastic cells, remain unknown. Therefore, the influence of NRF2 pathway activation on the growth of preneoplastic cells was assessed by studying the effect of SFN on the development of preneoplastic lesions in the rat liver. Six-week-old male F344 rats were given diethylnitrosamine (DEN), a genotoxic liver carcinogen, at a concentration of 10 ppm in drinking water or administered given furan, a non-genotoxic liver carcinogen, at a dose of 8 mg/kg body weight (5 times/week) by gavage for 13 weeks. One week after the last carcinogen treatment, the animals were given SFN at a concentration of 0 or 1200 ppm in diet for 6 weeks. All animals were then sacrificed and the livers were sampled for quantification of glutathione S-transferase placental form (GST-P) positive foci. The number and area of GST-P positive foci in the DEN + SFN group were significantly increased compared to those of the DEN alone group. There were no significant differences in this parameter between furan-treated groups. These data suggest that exogenous stimulation of the NRF2 pathway promotes the development of preneoplastic lesions induced by genotoxic carcinogens.

Poster Abstracts

P25: Immunohistochemical characterization of spontaneous lymphomas in Sprague-Dawley rats: comparison with the human counterpart

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Malignant lymphoblastic diseases are a heterogeneous group of neoplasms that arise from reticulo-endothelial and lymphatic systems. Based on the cell lineage, a lymphoid, myeloid and histiocytic type can be identified. The WHO/REAL human classification divides the numerous forms of neoplastic lymphoid disorders into 3 broad categories: B cell lymphomas, T/NK-cell lymphomas and Hodgkin's disease. Hodgkin's lymphoma represents about 30 % of all lymphomas. The remaining 70 % are generally designated by the term "Non-Hodgkin's lymphoma" which includes a very heterogeneous group of neoplasms that comes from malignant degeneration of various different immune system cells with 25–35 % of these neoplasia involving extranodal sites. To achieve the pathological diagnosis and to obtain a targeted and effective therapy, immunohistochemical studies are performed to discriminate between the various types of lymphoma. A panel of antibodies against proteins specifically expressed by B or T cells is usually chosen to establish the cellular origin of the neoplasia (e.g. the B immunophenotype express markers like CD23, CD5, CD10, CD43, bcl-6 and CD79a, while T cells can be tracked with the reactivity to CD3, CD4 and/or CD8 proteins). Experimental animal models that reproduce situations occurring in humans are commonly used to better study some diagnostic and biomolecular features. The colony of Sprague-Dawley rats used by the CMCRC/IR for more than 40 years has been shown to have a susceptibility to cancer which is very close to the human counterpart. Referring to the RITA nomenclature, internationally adopted for experimental toxicological studies in rodents (rats and mice), we classified lymphomas as lymphoimmunoblastic, lymphoblastic and lymphocytic; a comparison of the similar human / animal pattern are here presented. Haemolymphoreticular neoplasias are the most frequent malignant neoplasias in our historical controls (males 19 %; females 14 %). The experimental approach to demonstrate malignancy in lymphoid disease is based on the fact that cancer growth is clonal, in contrast to the non-clonal expression in reactive hyperplasia. In order to characterize the immunohistochemical pattern of these neoplasms specific markers are available for each cell line: the B phenotype can be investigated through the expression of Bcl-2, Bcl-6, CD45, CD79a; the T phenotype may be described using anti-CD3, anti-CD4, anti-CD8 antibodies, while the nuclear enzyme TdT can be investigated in early lymphoid cells. Our main interest is to characterize lymphomas and confirm pathological diagnosis, particularly when lesions are localized in extranodal site such as the lung. Since 40 years the CMCRC/IR laboratory has mainly used 70 % alcohol as a fixative, therefore we established a protocol based on a post-fixation procedure which permits to apply standard IHC protocols on both alcohol-fixed paraffin-embedded (AFPE) slices and formalin-fixed paraffin-embedded (FFPE) samples. Spleen from Sprague-Dawley rats has been selected as demonstrative tissue because the different cell lineages are present in this organ. Antibodies to PAX5, CD3, CD68 and Ki-67 were tested. In the AFPE slices, IHC staining results were comparable to the FFPE sections for sensitivity, specificity and intensity of the staining. The comparison of results on normal spleen tissue is presented in this poster and the optimization of the method for tissues with a diagnosis of hemolymphoreticular neoplasia is currently in progress, the preliminary results should be available at the date of the congress. In studies of chemical carcinogenesis, additional IHC markers can be used to help to distinguish lymphoid proliferation from neoplasias and determine the histotype. This is relevant to confirm diagnosis based solely on HE morphological analysis.

Poster Abstracts

P26: *In vivo* visualization of Pan-FGFR inhibitors mediated bone remodeling in rats by Osteosense®, a potential novel screening method

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It is reported that Fibroblast Growth Factor Receptor (FGFR) inhibitors induce physeal hypertrophy/dysplasia, changes in calcium and phosphorus plasma levels and result in generalized soft tissue mineralization (Ref. Brown et al.).

Several well established imaging techniques were used to visualize the physeal hypertrophy/dysplasia *in vivo*: Radiography (rats and dogs) and DEXA scans (dogs). Additionally, a more novel technique, a NIR bone-turnover imaging agent Osteosense® (rats) was evaluated to compare several compounds in discovery for their effect on bone remodeling at effective doses. Osteosense® targets and binds with high affinity to hydroxyapatite (exposed during bone turnover), and enables the *in vivo* detection and monitoring of skeletal changes that occur during either bone growth or bone resorption.

Methods:

Male *Nude (Crl:NIH-Foxn1^{rnu})* and Sprague Dawley *(Crl:SD)* rats, aged 4–6 weeks of age (obtained from Charles River, Germany), were treated twice daily with a pan-FGFR inhibitor or vehicle control by oral administration for 6 days. On days 5 and 6, rats received an intraperitoneal (i.p.) dose of a 680 nm NIR bone-turnover imaging agent (3 nmol/rat Osteosense®; PerkinElmer). On day 7, all rodents were anesthetized (2–3% isofurane) and imaged on IVIS® system (excitation 680 nm, emission 700 nm; PerkinElmer). After imaging, the animals were sacrificed and various tissues were taken for histopathology to assess any soft tissue mineralization and to evaluate the extent of the bone changes.

Results:

An increase in the Osteosense® incorporation in the knee regions, sternum and teeth of the rats treated with the pan-FGFR inhibitors was observed. Similarities were observed between rat strains, and this correlated well with location and severity as measured by the classical histopathology, in both these studies and 2 week repeated dose studies. The histopathological bone changes were characterized by physeal hypertrophy/dysplasia (H&E, Movat Pentachrome stain) , cartilage dysplasia of sternal synchondroses, (subphyseal) hyperostosis and remodeling changes, composed of prominent osteoclasts and atrophy of bone trabeculae with presence of erosion lacunae (Goldner stain). The bone remodeling effects were significantly reduced by lowering the dose given, while still retaining efficacy.

Based on the increased Osteosense® incorporation in the incisors, their histological evaluation was incorporated in all subsequent studies.

The incisors showed degeneration of odontoblasts and dentin dysplasia with presence of cellular debris and pronounced incremental lines. In mechanistic intermittent dosing studies, incisors showed an increase in accentuated incremental lines (due to disturbances in the mineralization process) and their number corresponded with the number of doses the rats received.

Conclusion:

In vivo imaging of physeal hypertrophy/dysplasia with the NIR bone-turnover imaging agent Osteosense® could be used to monitor bone remodeling changes. It might also add value in the screening of compounds with skeletal effects in discovery and for the evaluation of alternative dosing schedules.

Ref. Brown et al. Cartilage Dysplasia and Tissue Mineralization in the Rat Following Administration of a FGF Receptor Tyrosine Kinase Inhibitor. Toxicol Pathol 2005 33: p. 449–455

Poster Abstracts

P27: The possible protective role of vitamins C and E in fluoride intoxicated rats

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Introduction:

Fluoride toxicity occurs through certain conditions. Most cases of toxicity are caused by accidental ingestion of materials containing fluoride. Chronic exposure to fluoride causes illness in the form of fluorosis, not only in humans but also in domestic animals. The primary manifestation of fluorosis is mottling of teeth (dental fluorosis) and osteosclerosis (skeletal fluorosis). Besides these, non-skeletal fluorosis (toxic effects of Fluoride on soft-tissue or organ), gastro-intestinal disturbances, neurological disorders, reproductive dysfunctions and teratogenic effects have been reported in man and animals. The prevalence and severity of these chronic effects are related to the amount, duration of exposure and frequency of Fluoride intake. Aim of this study was to assess the protective effect of vitamin C and E in fluoride intoxication through evaluation of the histopathological changes in rat oral mucosa, teeth and jaw bone as a result of fluoride poisoning and .

Material and method:

The study was carried out on 45 adult male albino rats allocated into four groups treated for 8 weeks. Group I-Control (15 rats) was subdivided into three sub-groups (five rats each): negative control group (receiving distilled water), vitamin C control group (receiving 50 mg/kg/day vitamin C orally) vitamin E control group (receiving 3 mg/rat/day vitamin E orally). Group II-Fluoride treated rats (10 rats) orally received sodium fluoride dissolved in distilled water at a dose of 12mg/kg. Group III-Fluoride treated rats given vitamin C (10 rats). Group IV-Fluoride treated rats given vitamin E (10 rats). The oral mucosa, teeth, and jaw bone of the animals were processed for microscopic examination after sacrificing rats under ether anesthesia.

Results:

Regarding tooth, fluoride only group showed incremental lines in enamel and several thin alternating layers of remineralization and demineralization in dentin with bands of interglobular dentin. These findings were less severe in Fluoride group with Vitamin C while in Fluoride group with vitamin E there were a thin layer of remineralization of outer surface of enamel and well-formed alternating layers of remineralization and demineralization of dentin with no band of interglobular dentin. In Bone, fluoride only group showed resting lines of bone formation, increased osteoblast cells at the periphery and excessive chronic inflammatory cells. Compared to this last group fluoride group with Vitamin C showed increased resting lines of bone formation, less osteoblast cells at the periphery and some acute inflammatory cells while fluoride group with vitamin E intake showed only some resting lines of bone formation, few osteoblast cells at the periphery and few acute inflammatory cells. Fluoride only group showed atrophy of mucosal epithelium and increased musculature in the underlining connective tissues of oral mucosa. Compare to this group, atrophy of surface epithelium was less severe in Fluoride group with Vitamin C. Severe hyperplasia of surface epithelium was seen in Fluoride group with Vitamin E. It was concluded that vitamin C reduces the adverse effect of fluorides on soft tissue, while vitamin E, which prevents excessive accumulation of fluorides in bones and teeth, protects these tissues from fluorosis. Therefore, it seems that combined application of both compounds would be optimal for the prevention of the adverse effects of chronic fluoride intoxication.

Poster Abstracts

P28: Similar expression change of midline 1 on neuronal progenitor cells between developmental and adult-stage hypothyroidism in the hippocampal dentate gyrus of rats

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We have shown different impact on neurogenesis in contrast to similar distribution changes of GABAergic interneurons in the hippocampal dentate gyrus between the developmental and adult-stage hypothyroidism induced by exposure to methimazole (MMI) in rats. We found a sustained reduction of paired box 6-positive stem or early progenitor cells and a transient reduction of doublecortin-positive late-stage progenitor cells after the developmental hypothyroidism with MMI, while these cells were unchanged in the adult-stage hypothyroidism. In another study of developmental exposure to manganese (Mn) in mice, we identified *Mid1* showing hypermethylation of the promoter region in the dentate gyrus of offspring suffering permanent disruption of neurogenesis targeting late stage granule cell lineage. It is known that midline 1 is involved in left–right determination with a mutually repressive relationship between Shh in chick embryo. In the Mn-exposure study, midline 1-positive cells distributed in both of the hilar interneurons and subgranular zone (SGZ) progenitor cells with right side predominance in untreated controls; however, Mn-exposure canceled this asymmetry through to the adult stage. In the present study, we examined the cellular distribution of midline 1-expressing cells in the dentate gyrus after developmental and adult-stage hypothyroidism induced by MMI-exposure in rats. Exposure to MMI at 50 and 200 ppm in the drinking water was performed using pregnant rats from gestation day 10 to postnatal day (PND) 21 (developmental hypothyroidism) and adult male rats from PND 46 to PND 77 (adult-stage hypothyroidism). Offspring with developmental hypothyroidism at PND 21 or PND 77, and animals with adult-stage hypothyroidism at PND 77 were immunohistochemically examined. As well as in mice, rats also exhibited expression of midline 1 in the SGZ progenitor cells with right side predominance in untreated controls. By both developmental hypothyroidism and adult-stage hypothyroidism, midline 1-positive cells significantly decreased with MMI at both 50 and 200 ppm, and MMI-exposure canceled this asymmetry through to the adult stage in the developmental hypothyroidism. Interestingly, on PND 21, sonic hedgehog (Shh) expressed in the hilus and SGZ with left side predominance in untreated control offspring, while by developmental hypothyroidism, Shh-positive cells significantly increased with MMI at 200 ppm and the asymmetry was also canceled by MMI-exposure. These results suggest that developmental hypothyroidism causes permanent cancellation of bilateral difference of neurogenesis probably through disruption of epigenetic gene control of midline 1-expression. Such risk also could appear in progenitor cells by adult-stage hypothyroidism. Abnormal expression of midline 1 following MMI-exposure may contribute to aberrant distribution of Shh-positive cells.

Poster Abstracts

P29: Intramuscularly injectable long-acting drug microsuspensions: Histopathological exploration of the local *in vivo* disposition and the implications for drug release

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Introduction:

The complexity of the local *in vivo* disposition of sustained-release formulations for intramuscular (IM) administration has not been investigated thoroughly so far. The fate distribution of IM injected drug microparticles might substantially modulate the *in vivo* pharmacokinetics.[1]

Objectives:

The aim of the present study was to obtain a deeper understanding of the distribution of IM injected crystalline drug microparticles as well as their possible interactions with inflammatory cells in rats and the potential effects on the pharmacokinetics.

Experimental conditions:

A long-acting injectable crystalline suspension ($d_{v,50}$ = 1.18 µm) of a poorly water-soluble ester prodrug was injected intramuscularly *(m. biceps femoris)* in adult male Wistar rats (n = 3 per time point). An identical vehicle control solution free of solid particles was administered into the contralateral hind legs of all rats. Histopathological and immunohistochemical evaluation of the administration sites and draining lymph nodes were performed using bright field and polarized light microscopy 2 h, 4 h, 8 h, 24 h, 48 h, 72 h, 1 week, 2 weeks, 3 weeks, 4 weeks and 8 weeks after injection. Plasma aliquots were taken at each time point and the concentration of (hydrolysed) active moiety was determined by LC-MS.

Results and discussion:

Following IM injection, the microsuspension appeared to form a highly concentrated depot, primarily localized in the interfascial spaces. Histopathological assessment of the IM administration site revealed the onset of a chronic granulomatous inflammatory reaction after 48 h despite the lack of macroscopic evidence of inflammation in the intact rat hind leg. Granulocytes and to a lesser extent $ED1^+$ macrophages of vascular origin were visible at the periphery of the formulation depot as early as 2h after the injection. Beyond 24 h, a central cell-free region consisting of the drug microsuspension depot, surrounded by an infiltrating front of epitheloid macrophages and granulocytes, could be observed. Depending on the time point, this layer of inflammatory macrophages was lined by a mixture of granulocytes, fibroblasts, histiocytes and some lymphocytes and plasma cells. The macrophages were loaded with microparticles as evidenced by their swollen and granulated appearance under bright field microscopy and the birefringence observed with polarized light microscopy. The first capillary sprouts ($CD31^+$) could be detected 24h after dosing. After 72 h, a pronounced radial angiogenesis was observed. The ipsilateral iliac lymph nodes did not contain birefringent particles, but a positive Oil Red O staining, suggesting accumulation of dissolved prodrug or palmitic acid, was detected. The plasma concentration-time profiles showed an atypical triphasic absorption phase (T_{max} = 192 h) followed by a log-linear elimination.

Poster Abstracts

Conclusion:

An extensive inflammatory reaction resulting in intracellular particle accumulation in epitheloid macrophages was observed after IM injection of a long-acting crystalline microsuspension in the rat. This might drastically influence the current understanding of the IM drug release kinetics, as evidenced by the altered pharmacokinetics.

Acknowledgments:

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References

[1] Hill A. *et al.* (2013) – *J. Control. Release 168 (2013): 77–87*

Poster Abstracts

P30: FcRγIV and Clec4a3: novel markers of activated macrophages in mice

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Mice treated with compound X displayed activated macrophages in different organs (lymph nodes, jejunum). Genomic expression profiling revealed high mRNA expression of FcγRIV and Clec4a3

FcγRIV is a γ chain-dependent, activating IgG Fc receptor, known to be expressed on myeloid cells. This Fc receptor binds with intermediate affinity to mice IgG2a and IgG2b, which both show vast protective and pathogenic properties.

Clec4a3 is a member of the C-type lectin receptor family. This large receptor family comprises specialized receptors utilized by myeloid cells.

FcγRIV expression was assessed by immunohistochemistry (IHC) and was highly expressed in tissue macrophages. Due to the lack of specific commercial available Clec4a3 antibodies, in-situ hybridization (ISH) was applied for localization. High expression was confirmed by ISH on the same type of myeloid cell. Both, IHC for FcγRIV and ISH for Clec4a3 revealed a prominent positive staining of activated macrophages with staining intensity being superior compared to the conventional markers of macrophages (F4/80, CD68, CD11b), facilitating detection of activated macrophages in different organs. Hence we propose FcγRIV and Clec4a3 as novel markers for monitoring tissular macrophage activation in mice.

Poster Abstracts

P31: Optical Coherence Tomography (OCT) and Fluorescein Angiography as a useful screening tool for ocular toxicity

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Introduction:

Ocular toxicity can be induced by many systemic drugs and several drug-induced ocular side effects have been found in clinical studies. However, it is difficult to detect ocular toxicity in preclinical studies due to lack of appropriate evaluation methods.

Optical coherence tomography (OCT) and fluorescein angiography are useful imaging tools in that they provide real time images during a study period, while histopathology gives an image only after sacrificing animals. Using OCT and fluorescein angiography besides histopathology, we tried to find effective approaches, which can be used in preclinical studies, to screen drug-induced ocular toxicity before trial to human.

Methods:

Two male Cynomolgus monkeys were orally administered two different MAPK/ERK kinase (MEK) inhibitors, A-001 and Z-001, respectively, which are promising anticancer drugs and known to induce ocular side effects.

One monkey was administered A-001 for 12 days, and the other animals was administered Z-100 for 8 days (found dead on day 9). For ophthalmic examinations, OCT (Heidelberg Engineering OCT SPECTRALIS TR-KT-327) on day 0 (pre-dose), 5, 7, and 12, fluorescein angiography (TOPCON RETINAL CAMERA TRC50IX) on day 12, and histopathology were conducted.

Results:

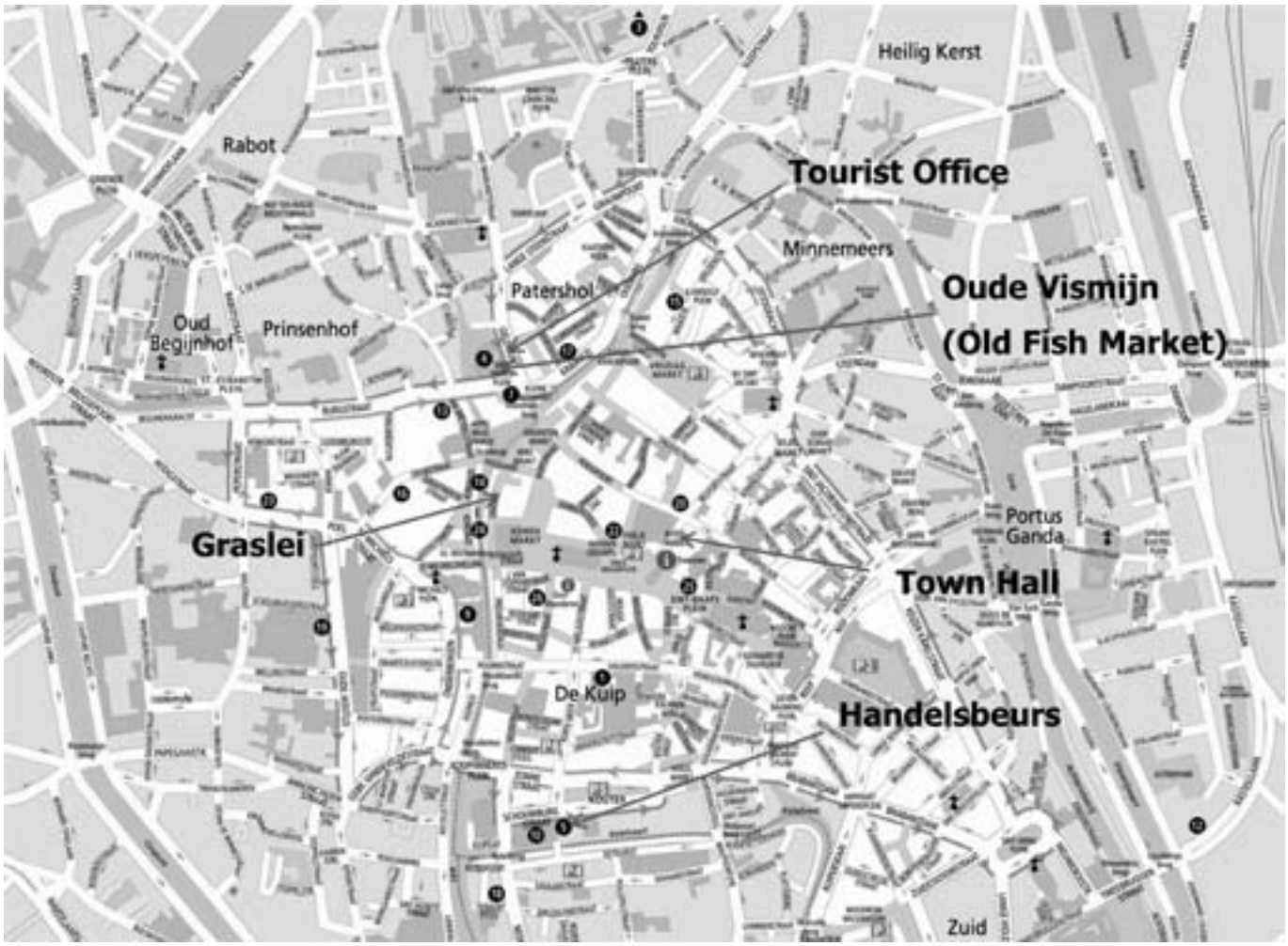
On day 7, central serous chorioretinopathy (CSCR), a known side effect of MEK inhibitors, was identified in one treated with Z-100 by OCT imaging.

The other treated with A-001 showed no ocular changes by OCT and fluorescein angiography.

Histopathology wasn't useful to detect edematous change because layers of eye were separated during processing the tissues.

Conclusion:

Thus, OCT and fluorescein angiography are considered to be useful tools to detect ocular toxicity that is not able to be detected by histopathology.



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