



Session 7.2

Innovative approaches for alternative methods development

Poster

Study on cytotoxicity assay and fluorescence probe on *in vitro* sensitisation assay using h-CLAT (human Cell Line)

Takao Ashikaga, Sakiko Sono, Kasturako Yoneyama and Hiroshi Itagaki

Shiseido, Safety and Analytical Research Center, Yokohama, Japan

In this study, several kinds of cytotoxicity assays and fluorescence probes were examined in the h-CLAT. In the first protocol, MTT assay was performed to determine the IC_{50} as a basis for dose setting, and then cell viability was measured by PI assay when CD86/CD54 expression was evaluated. Now we use PI assay for both dose setting and actual test. Four kinds of cytotoxicity assays (MTT, PI, FDA and 7-AAD) were compared using four chemicals (three allergens, DNCB, Ni, pPD, and one non-allergen, SLS). In the cases of Ni and pPD, the presence of the chemicals in the culture medium influenced the results of MTT assay. pPD influenced the results of FDA assay due to its intrinsic fluorescence. On the other hand, the IC_{50} s of the four chemicals were almost unaffected in the cases of both PI and 7-

AAD assay. Therefore, these two assays were thought to be suitable for this test. FITC-labeled antibodies are used in the h-CLAT and showed high prediction. PE, another kind of commercially available fluorescence probe, was evaluated using DNCB, SLS and Tween 80 (a non-allergen). Previously these three chemicals were discriminated actually by FITC-labeled antibody. In the case of DNCB, the augmentation of CD86 stained with PE-labeled antibody was higher than that obtained with FITC. In the case of SLS, no augmentation was observed when using either FITC or PE-labeled antibodies. However, for Tween80, augmentation of CD86 was observed only when PE-labeled antibody was used. Further study will need on the usefulness of PE.



Poster

Applying the Three Rs to mouse mutagenesis studies

Nirmala Bhogal

FRAME, Nottingham, UK

Mouse mutagenesis studies are either phenotype-driven or genotype-driven. The former are reputedly more relevant with regards genomic annotation and methods have traditionally involved inducing mutations using chemicals in live animals. Such methods rely heavily on germline transmission to any offspring. Generally, mutation rates are low and germline transmission poor with many of the offspring not surviving to reproductive age or being sacrificed or subjected to invasive genotyping. As chemical mutations occur randomly, it is not uncommon for many mice to have been used without a single new strain being found. Transgenic methods, although gene-specific, are also very inefficient. Recent developments in the field of embryonic stem cell manipulation have resulted in higher mutation and transmission rates and the use of less invasive genotyping protocols. RNA interference and gene induction methods allow

genomic annotation based on transient rather than permanent genetic defects. However, the application of such techniques relies heavily on understanding the interplay between genes and their expression products. Furthermore, without appropriate information management, it is difficult to assess the relevance of mouse mutagenesis studies to human health. The sequencing of the human genome and the discovery of DNA variations, including single polynucleotide polymorphisms, may facilitate discovery of biomarkers of toxicity and disease susceptibilities from genotyping and phenotyping studies with human volunteers and from genomics-based research. The significance of biotechnology, bioinformatics and human population research to the Three Rs and the development of alternatives to mouse mutagenesis studies will be reviewed.

Poster

Changes induced by heat shock in *in vitro* culture of gastrulating mammalian embryo- application of FTIR and RAMAN spectroscopy

Floriana Bulic-Jakus¹, Ana Katusic¹, Maja Balarin², Maja Vlahovic¹, Ozren Gamulin², Gordana Juric-Lekic³ and Jasminka Brnjas-Kraljevic²

¹ School of Medicine, University of Zagreb, Department of Biology, Zagreb, Croatia; ² School of Medicine, University of Zagreb, Department of Physics, Zagreb, Croatia; ³ School of Medicine, University of Zagreb, Department of Histology and Embryology, Zagreb, Croatia

An original organ culture model of the rat gastrulating embryo-proper was used to investigate teratogenic effects of a severe heat shock. Additionally, FTIR and RAMAN spectroscopy were applied to detect possible changes in composition of the culture medium.

Embryonic parts of 9,5-days-old rat embryos were cultivated for two weeks at the air-liquid interface in serum-supplemented Eagle's MEM (50%). Heat shock (43°C) was applied for 24 hours and embryos were transferred to 37°C. Controls have spent the 14-day culture period at 37°C. Culture medium was changed every other day. Culture medium as well as culture conditioned medium was frozen at -20°C. Two diameters of explants were measured several times by an ocular micrometer and compared by t-test. FTIR and FT-Raman spectroscopy were

done on defrosted and dialysed culture media. The infrared spectra in transmission mode were recorded using Perkin Elmer GX spectrometer and Raman spectra with Raman modul of same spectrometer. The changes in spectra of heat shocked and control group were followed in region of 1800 cm⁻¹ to 600 cm⁻¹ which is the region of active vibrations of biological macromolecules.

The diameters of heat shocked embryos were always significantly smaller than in controls ($p < 0,01$) which showed that the growth of embryos was impaired by severe heat shock. FTIR and RAMAN spectroscopy showed comparable results. It seems that either of these complementary spectroscopical methods may serve as a suitable method for a quick assessment of the impact of extraneous factors on a complex biological system.



Lecture

A novel strategy applied on hepatocytes allowing to mimic *in vitro* the human metabolic idiosyncrasy

Jose Castell¹, Augustin Lahoz², Daniel Henández¹, Maria J. Gómez-Lechón¹ and Ramiro Jover¹

¹Hospital Universitario La Fe, Unidad de Hepatología Experimental, Valencia, Spain;

²Advancell S. L. Barcelona, Barcelona, Spain

Drug metabolism is a key determinant of drug pharmacokinetics variability in human beings. Well-recognised causes of such interindividual differences are phenotypic as well as genotypic differences in the expression of the enzymes involved in drug metabolism. Indeed, a major characteristic of CYP enzymes is the large range of interindividual variability in the expression of enzyme protein. Variable CYP activities are important sources of variability in the response (effect *vs.* side effects) to many drugs.

Genotypic variability is easy to identify by means of polymerase chain reaction-based or DNA chip-based methods, whereas phenotypic variability requires direct measurement of enzyme activities in liver, or, indirectly, measurement of the rate of metabolism of a given compound *in vivo*. There is a great deal of phenotypic variability in human beings, only a minor part

being attributable to gene polymorphisms. Enzyme activity measurements in a series of human livers, as well as *in vivo* studies with human volunteers, show that phenotypic variability is, by far, much greater than genotypic variability. The sources of such variability are many, including diet, age, disease and exposure to a variety of environmental factors, including smoking.

Reproducing *in vitro* the variability and metabolic idiosyncrasy of human beings has been hampered by the considerable difficulty in governing simultaneously the expression of several genes in hepatocytes by conventional molecular biology tools. By the use of suitable viral expression vectors encoding all major drug metabolising enzymes, we have succeeded in generating cells that can virtually reproduce any human phenotype providing a valuable tool to investigate the role of idiosyncrasy in drug metabolism and toxicity *in vitro*.

Lecture

Development of a novel diagnostic ELISA for human insulin using serum-free cell culture

Megha S. Even¹, Chad B. Sandusky¹, Neal D. Barnard¹, Jehangir Mistry² and Madhur K. Sinha²

¹PCRM, Research Dept, Washington DC, USA; ²Linco Research, Inc., St. Charles, MO, USA

Background: Concerns for animal welfare led us to develop a custom ELISA, for the measurement of human insulin, that replaced both the use of the ascites method and the use of fetal calf serum, while providing sufficient precision and reliability for clinical and research applications.

Methods: Insulin monoclonal antibodies were grown *in vitro* (derived from hybridoma cultures) in RPMI 1640/DMEM (1:1 v/v) supplemented to 4 mmol/l L-glutamine, 4% Maxi-Mab Mark II Supplement, and 2% Complex Lipid Solution. After weaning of the cells, fetal calf serum was not used for antibody production. A two-step ELISA was developed using recombinant human insulin (standard), charcoal-treated human serum (matrix), 50 mmol/l PBS containing human serum (assay buffer), and HRP-TMB detection system.

Results: The assay characteristics include sensitivity of 1.56 uU/ml, dynamic range of 1.56 to 200 uU/mL, no cross-

reactivity with human C-peptide or pro-insulin, intra- and inter-assay CVs of <10%, recovery of exogenously added insulin to plasma samples of 102.2-105.7%, and linearity of dilution (1/2, 1/4, and 1/8) of insulin spiked plasma samples as 93-110% of undiluted plasma samples. Circulating insulin levels in ten healthy volunteers were measured using both conventional ELISA methods and our new ELISA with absolute values similar between the two assays.

Conclusions: A highly specific and sensitive insulin ELISA was developed without using the ascites method or fetal calf serum for monoclonal antibody production. These methods could serve as a guide for reducing animal use for antibodies produced for other types of immunoassays and diagnostic tests.



Poster

***In vitro* model to compare surgical meshes in cell cultures**

Stefan Farke, Michael Duchrow, Sven Meeder, Matthias Friedrich, Hans-Peter Bruch, Thilo Wedel and Rainer Broll

University Clinic Schleswig-Holstein, Campus Luebeck, Surgery, Luebeck, Germany

Purpose: Regarding the recently demonstrated different effects of surgical polypropylene meshes on different cell lines, we established an *in vitro* model to compare surgical meshes without animal use.

With this model we examined different meshes with resorbable and non resorbable filaments. Some meshes were examined after resterilisation by autoclaving at 121°C for 20 minutes.

Methods and Materials: A variety of surgical meshes were tested in our model. Parts of the meshes (1 cm²) were sterile incubated with human cells for 72 hours. Apoptotic and proliferation index were measured. Scanning electron microscopy was carried out on the mesh-samples.

Results: With this *in vitro* model we could demonstrate some differences between the examined meshes. We could measure

differences in the apoptotic index and proliferation index as well. In some meshes the apoptotic index was significantly increased while another one did not alter this index.

Scanning electron microscopy demonstrated alteration of the filament structure in all meshes after incubation with cell cultures.

Discussion and conclusion: Surgical implants are normally tested using animals prior to introduction into clinical use. With our model we demonstrate differences in the effect of the meshes on human cell lines. The apoptotic effect was different between the meshes, in all meshes the incubation with cell cultures lead to alteration in scanning electron microscopy pictures.

In our opinion this *in vitro* model can serve as a pre-clinic model to compare surgical meshes in view of biocompatibility and can lead to a reduced use of animals.

Poster

Assessment of infectivity of cryptosporidium oocysts by Fluorescent *In Situ* Hybridisation (FISH) as an alternative method to mouse bioassay

Thaddeus K. Graczyk

Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health Sciences, Baltimore, USA

Introduction: Cryptosporidium is a waterborne pathogen which significantly contributes to mortality of immunosuppressed people. Over 75% of AIDS patients die due to cryptosporidiosis as there is no treatment for this disease. Diarrheal disease is initiated by a microscopic size transmissive stage (i.e., 5 Fm), the oocyst.

Methods: Infectivity of Cryptosporidium is routinely assessed by mouse bioassay which uses large numbers of neonatal mice for challenge with Cryptosporidium; it is followed by animal sacrifice, necropsy, and histological analysis. Mouse bioassays have several serious deficiencies in addition to having to use large numbers of animals. Therefore, we propose a new molec-

ular method for assessment Cryptosporidium infectivity, i.e., Fluorescent *In Situ* Hybridisation (FISH). This method will eliminate the need for the mouse bioassay by the U.S. and world-wide laboratories concerned with Cryptosporidium and cryptosporidiosis.

Results and Discussion: Our preliminary data indicates that the FISH method is superior to the mouse bioassay because it is several-fold more sensitive and specific, provides visual color-coded information on pathogen morphology, has extraordinarily high detection threshold, i.e., single pathogen vs 10³-10⁶ pathogens in mouse bioassay, and is easy to use and cost-and-labor effective, i.e., \$5 vs \$40 and 1.5 hr vs 10 days, respectively.



Poster

Detecting neurotoxicity through electrical activity changes of neuronal networks on microelectrode array neurochips

Alexandra Gramowski¹, Simone Stüwe¹, Frank Kletzin², Dietmar Schiffmann¹, Konstantin Jügel¹, Jan Loock³, Guenter W. Gross⁴, Olaf Schröder⁵, Elke Thedinga⁶ and Dieter G. Weiss¹

¹University of Rostock, Institute of Biological Sciences, Rostock, Germany; ²University of Rostock, Clinic for Anaesthesiology, Rostock, Germany; ³University of Rostock, Clinic for Internal Medicine, Rostock, Germany; ⁴University of North Texas, Center for Network Neurosciences, Denton, TX, USA; ⁵Pattern Expert, Borsdorf, Germany; ⁶Bionas GmbH, Rostock, Germany

Neuronal network cultures respond to transmitters, their blockers, and other neurotoxic compounds in a substance-specific manner. Networks grown on 64-microelectrode neurochips remain spontaneously active and stable for many months providing a suitable test platform. This hybrid system of cells and microelectrodes forms a sensing device based on quantitative analyses of the complex signal patterns of living neuronal networks. High-content screening is a substantial improvement for detecting undesired effects of test compounds on neuronal activity at an early phase of drug development.

With several examples of neurotoxic, sedative and narcotic substances we underline the suitability of this test system. Experiments with the neurotoxic antifungal and antifouling compound trimethyltin chloride show that spinal cord and auditory cortex cultures exhibit characteristic and dose-dependent changes of their electrical firing patterns (Gramowski 2000,

NeuroToxicology 21). Data derived from dose-response curves for the anaesthetic ketamine confirm its strong receptor-specific effects on the electrical activity. Employing refined pattern recognition analyses we demonstrate that it will be possible to ascribe the network impairment to different receptors and ion channels. This is shown for strychnine, bicuculline and picrotoxin which bind to the glycine and GABA-A receptors respectively.

These sensitive and quantitative (Gramowski 2004, *Eur. J. Neurosci. 19*) responses have triggered strong interest in using such platforms as broadband biosensors for screening for adverse side effects of various classes of compounds during early drug development and at the same time as a means for reducing *in vivo* animal tests.

Supported by State of Mecklenburg-Vorpommern and European Community (ERDF).

Lecture

Cell array-based RNA interference as an alternative to the genetically modified animal experimentation

Michael Janitz¹, Dominique Vanhecke¹, Alexander Scheffold² and Martin Weber³

¹Max Planck Institute for Molecular Genetics, Berlin, Germany; ²German Rheumatism Research Center, Berlin, Germany;

³Qiagen GmbH, Hilden, Germany

Loss-of-function studies using knock-out mice and gain-of-function studies using transgenic mice are a well-established approach to study gene function in *in vivo* situation. Besides undisputed contribution in the field of functional genomics these techniques intrinsically require a large number of animals for the production of the gene-manipulated animals as well as for the testing of gene function in the various physiologic cell populations. This requirement raises ethical issues concerning experimentation on animals, but it is also expensive due to the high cost of animal maintenance. Therefore, there is a clear need for development and optimisation of *in vitro* technologies, which could contribute to reduction, if not replacement, of animals in the existing knockout and transgenic mice technology.

In the present study a development of a high-throughput *in vitro* experimental approach, the transfected cell array (TCA), in primary mammalian cells as an alternative to generation of genetically manipulated animals for loss-of-function as well as for gain-of-function studies will be presented. For that purpose we applied the RNA interference (RNAi) technology, which allows a specific inactivation of target genes. Thus, genes functions in various cell types could be investigated. The array-based method allows for high throughput functional analysis of hundreds of genes with the minimal cell number requirements. Moreover, usage of primary cells implies that experimental results can be directly transferred into *in vivo* situation in animal models and man.



Poster

Toxicity of nanoscale cationic polymers *in vitro* and *in vivo*

Anne Kahru¹, Monika Drews¹, Lee Pollumaa¹, Kaja Kasemets¹, Toomas Veidebaum² and Priit Kogerman¹

¹National Institute of Chemical Physics and Biophysics, Molecular Genetics, Tallinn, Estonia;

²National Institute for Health Development, Research Centre, Tallinn, Estonia

The toxicity issues concerning nano-scale compounds are constantly gaining more attention. Lack of inherent toxicity is of strategic importance if nanomaterials are planned for medicine. Nanoparticles (e.g. dendrimeric macromolecules, cationic polymers, functionalised fullerenes) are widely studied for their use in targeted drug delivery, particularly drugs based on proteins, DNA and RNA. We have analysed the toxicity of two cationic polymers: poly(amidoamine) PAMAM G5 dendrimer (Mwt ca 25,000) and branched polyethyleneimine (PEI, Mwt ca 25,000) to biological systems of different complexity (a test battery). Different acute toxicity endpoints: the 30-minute inhibition of light output of photobacteria *Vibrio fischeri*, 24 h impairment of growth of protozoa *Tetrahymena thermophila*, 24 h viability of human cell line K562 (trypan blue exclusion) and mortality of

mice (i.p.) were evaluated. In all tests dendrimer was 2 10-fold less toxic than PEI. For the *in vitro* test the average acute toxicity of PEI and dendrimer were 18 and 180 mg/l, respectively. The acute toxicity of PEI to mice was 74 and that of dendrimer 150 mg/kg. The 24 h IC₅₀ for K562 cells and 30 min EC₅₀ for photobacteria were practically similar: 25 mg/l for PEI and about 270 mg/l for dendrimer, showing the potential of rapid ecotoxicological tests in toxicity screening of nanoparticles. In addition, the study of the adverse effects on different biological organisational levels helps to discover the mechanisms of toxicity of these emerging chemicals and to predict their hazard to ecosystems as the production of some nanomaterials is already in high production volume scale.

Poster

Quantitative evaluation of activity of osteoclasts derived from peripheral blood precursors

Iwona Kochanowska¹, Ryszard Kozłowski², Krzysztof Włodarski³, Andrzej Wojtowicz⁴, Dariusz Kalinowski⁴, Dariusz Sładowski⁵ and Kazimierz Ostrowski^{*3}

¹Polish Academy of Sciences, Institute of Immunology and Experimental Therapy, Wrocław, Poland; ²The Regional Centre of Haematology, Wrocław, Poland; ³Medical University of Warsaw, Department of Histology, Warsaw, Poland; ⁴Medical University of Warsaw, Department of Oral Surgery, Warsaw, Poland; ⁵Medical University of Warsaw, Department of Transplantology and Central Tissue Bank, Warsaw, Poland

Osteoporosis is nowadays the medical as well as sociological problem because of the pronounced progress of the loss of skeletal tissues found in high percentage of the population of postmenopausal women in Europe. The animal tests for experimental osteoporosis are cruel and unacceptable. Two populations of cells – osteoblasts and osteoclasts – are responsible for the balance and regulation of bone rebuilding process. The precursors of both kinds of these cells are found in bone marrow. Peripheral blood is used as the source of preosteoclasts differentiated *in vitro*.

The proposed methodology is the modified technique called “PITS”. This technique is based on direct action of osteoclasts

on thin bone plates *in vitro*. Osteoclasts seeded on the bone plate adhere to it, and form underneath a depression called “pit” thanks to the activity of their proton pump which leads to lowering of local pH. The original technique based on counting the pits is not accurate and reliable. Therefore we decided, to measure the activity of osteoclasts by biochemical technique of estimation of N- or C-telopeptides released by digestion of bone matrix by MMPs (matrix-metalloproteinases) secreted by osteoclasts. The increase of concentration of telopeptides is supposed to be specific marker of activity of osteoclasts. The whole process is observed in the tissue culture wells in which bone fragments and osteoclasts are incubated in proper medium.



Lecture

Assessing the toxicity of smoke derived from polymer combustion using human lung cells (A549)

Fatma Lestari¹, Anthony Green², Gautam Chattopadhyay³ and Amanda Hayes¹

¹University of New South Wales, Chemical Safety and Applied Toxicology Laboratories, Sydney, Australia; ²University of New South Wales, School of Safety Science, Sydney, Australia; ³University of New South Wales, School of Civil and Environmental Engineering, Sydney, Australia

Many synthetic polymers have been introduced in building materials and mass transport, which have resulted in the generation of more toxic and hazardous combustion products. An alternative method using *in vitro* techniques has been developed to assess the toxicity of smoke derived from polymer combustion on human lung cells (A549). The lung cells were grown on a porous membrane and exposed to the combustion toxicants at the air/liquid interface with a dynamic exposure method using the Harvard Naviocyte Chamber. A laboratory small-scale fire model using a vertical tube furnace was designed for the generation of combustion products. A range of building and mass transport materials, including PMMA (Polymethyl methacrylate), Polyethylene, Polycarbonate, Polypropylene, and PVC (Polyvinyl chloride) were investigated. Three *in vitro* methods were studied including: MTS (3-(4,5-dimethylthiazol-2-yl)-5-

(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), NRU (Neutral Red Uptake) and ATP (Adenosine Triphosphate) assays. The thermal degradation products were analysed using ATD-GCMS (Automatic Thermal Desorption – Gas chromatography Mass Spectrometry) method, coupled with a direct reading measurement using a CO/CO₂ sensor. IC₅₀ (50% inhibitory concentration) values were generated using this method, these included: PVC = 1.5-2.3 mg/L air; Polyethylene = 2.6-4.5 mg/L air; Polypropylene = 4.1-5.1 mg/L air; PMMA = 4.4-6.4 mg/L air; and Polycarbonate = 7.9-13.3 mg/L air. The toxicity rank of the polymers as determined using the three assays from most toxic to least toxic were: PVC>Polyethylene>Polypropylene>PMMA>Polycarbonate. The technique developed here has the potential to be an alternative method to the current fire smoke toxicity standard.

Lecture

RNA interference: A novel alternative approach in nephrotoxicity studies

Tara McMorow, Eric Campbell, Craig Slattery, Niamh Tuite and Michael Ryan

Conway Institute, Pharmacology Department, University College Dublin, Dublin, Ireland

One of the main goals in functional genomics has been the development of tools that allow easy manipulation of gene expression levels, that would be suitable for high through-put screening. RNAi has emerged as one of the preferred approaches to achieve this goal. It is an important biological mechanism in the regulation of gene expression in animals and plants.

Functional investigation of the complex regulation of molecular switches and their effectors are key to understanding nephrotoxicity. Nephrotoxicity is caused by several drugs such as immunosuppressive agents and is associated with the development of renal fibrosis. We have established several *in vitro* models of nephrotoxicity and have identified several key genes, using both micro-array and differential gene expression (SSH)

technology, which we believe are involved in this process. We have used RNAi to analyse the role of these genes in the development of nephrotoxicity. A number of interesting differentially expressed genes involved in signal transduction, cell cycle regulation and cytoskeletal dynamics were identified and are being examined further as potential therapeutic targets.

In conclusion we have demonstrated that silencing of key genes with RNAi has helped to elucidate their role in the development of renal fibrosis and nephrotoxicity. Further examination of these differentially regulated genes may lead to the identification of novel therapeutic targets and potential adjunct therapies. Finally we believe that the potential role of RNAi as an alternative to animal models is just beginning to be realised.



Poster

Biosensor-controlled perfusion cell culture: An innovative biomonitoring system potentially useful to supplement or partially substitute animal studies

Birgit Mraz¹, Peter U. Abel², Wolf-Dieter Jülich², Ulrike Lindequist² and Thomas von Woedtke¹

¹ Sensor Research Center, Greifswald, Germany; ² University of Greifswald, Institute of Pharmacy, Greifswald, Germany

Cell cultures are well established in active substance testing. In an open perfusion system cells are feeded continuously by nutrient medium and metabolic products are removed continuously. Because glucose is the main energy source, glucose consumption of cell culture can be estimated by an amperometric enzyme biosensor continuously and non-invasively. Kinetics of this metabolic parameter is used to characterise activity profiles of substances by an *in vitro* test system, under conditions closely approximated to the *in vivo* situation.

24 hours after the transfer of human amniotic epithelial cells (FL cells) into the perfusion system, a metabolic equilibrium is reached characterised by stable glucose consumption. It was significantly reduced during cell exposition to 30 mg/l hydrogen peroxide, occurring physiologically in the micro-environment of macrophages. Similar effects have been induced by low heavy

metal concentrations being significant as water pollutants (e.g. 390 µg/l copper), which opens the possibility for early prediction of disturbances of ecosystems [1, 2]. In search of new active substances, both kinetics of polio virus infection and protecting effects of natural antiviral substances were monitored [3]. Recently, stimulating activities of natural products on cell metabolism were demonstrated. Actually, the biosensor-controlled perfusion cell culture is on an experimental stage of test development, but has a great potential to be further evolved into a validated laboratory system to supplement and/or substitute animal tests.

[1] von Woedtke et al. (2002) *Med. Biol. Eng. Comput.* 40, 704.

[2] Fenske et al. *Int. J. Hyg. Environ. Health* (submitted).

[3] von Woedtke et al. (2002) *Pharmazie* 57, 270.

Poster

Comparison of tissue barriers – evaluation of the permeability *in vitro*

Heidi Nevala, Tarja Toimela and Hanna Tähti

University of Tampere, Medical school, Tampere, Finland

When developing new drugs it is important to investigate their passage to different tissues. Specific barriers regulate this permeability by forming tight intercellular junctions. The brain is protected by a blood-brain barrier (BBB) which is composed of brain microvessel endothelial cells. Similarly, the retina has a blood-retinal barrier that resembles the BBB. Due to a tight epithelial cell structure, the absorption of drugs through the small intestine is restricted as well.

The aim of this study was to compare the barrier integrity of primary porcine microvessel endothelial cells (PMEC), human retinal pigment epithelial cell line (ARPE-19), and human colonic adenocarcinoma cell line (Caco-2). Cells were grown on filter inserts, and the integrity of the barrier was evaluated with the measurement of trans-epithelial and trans-endothelial elec-

tric resistance (TEER). The cells growing on chamber slides were immunostained for a tight junction protein, occludin. The TEER of ARPE-19 cells appeared to be low when compared to PMEC and Caco-2 cells. Immunocytochemistry revealed the existence of occludin in all cell types studied.

In conclusion, PMEC and Caco-2 cells seem suitable for *in vitro* permeability studies, whereas ARPE-19 yielded less optimal TEER values. The characterisation of transport proteins and the inducibility of tight junctions in these cells is under investigation.

Acknowledgements: EDIT Project, TEKES (The National Technology Agency of Finland).

**Poster****Validation of various blood flow-meters in an artificial circuit as a 3R concept**

Suzanne Osorio-da Cruz, Mustafa Cikirikcioglu, Afksendyios Kalangos and Beat Walpoth

Hôpital Cantonal Universitaire de Genève, Département de Chirurgie Cardiovasculaire, Genève, Switzerland

Introduction: Intra-operative flow measurement during bypass operation is helpful to exclude technical failures. Blood flow-meters validation is mandatory to confirm reliability of measured results. Preclinical validation studies may be performed in the animal models and artificial circuits. The aim of this study is to evaluate the feasibility and the impact of artificial circuit tests before animal trials as part of the 3R concept.

Methods: The artificial circuit was constructed with PVC tubing sets, one roller pump and two reservoirs. Pulsatile flow was obtained with short length, small diameter tubes. Flow conditions were continuously controlled with invasive pressure and flow signals. A 6 cm long swine carotid artery segment was inserted in the circuit, which was filled with pig blood and immersed in a 37°C water bath. Swine blood and arteries were collected from euthanised animals from other studies. The fol-

lowing devices were tested: Quantix OR®, CardioMed® and Medi-Stim®. Time collected true flow amount was used as reference during each measurement. The following tests were performed with the artificial circuit: 1. Correlation and agreement analysis, 2. Device reproducibility and measurement stability, 3. User accuracy (intra- and inter-observer variability).

Results: All devices showed good results in the reproducibility tests, the correlation coefficients between flow-meters and time collected true flow being over 0.98 ($p=0.01$).

Discussion: The testing of blood flow-meters in the artificial circuit was reproducible. The use of artificial circuits is a useful previous step for animal studies facilitating device and probe design improvement, thus decreasing the number of animal trials.

Poster**Aquatic hazard assessment of petroleum products using biomimetic solid phase microextraction**

Thomas Parkerton

Exxon Mobil Biomedical Sciences Inc, Annandale, New Jersey, USA

Petroleum products often consist of multi-component hydrocarbons with variable composition. Despite this complexity, the constituent hydrocarbons typically act via narcosis in aquatic toxicity tests. Consequently, adverse effects are expected once the total molar sum of the individual hydrocarbons in organism lipid (i.e. Tissue Body Residue) exceeds a critical threshold (i.e. Critical Body Residue) corresponding to narcosis. Recent research has shown that solid phase microextraction (SPME) fibers provide a surrogate for organism lipid thereby providing a convenient analytical tool for estimating the TBR and predicting narcotic effects of complex mixtures. To investigate the applicability of this analytical tool for predicting the aquatic toxicity of petroleum substances, acute toxicity tests for an invertebrate (*Daphnia magna*), fish (*Onchorynchus mykiss*) and algae (*Pseudokirchneriella subcapitata*) were performed in parallel with SPME measurements for a model petroleum product

(No. 2 Fuel Oil). Total molar hydrocarbon concentrations on polydimethylsiloxane SPME fibers were quantified using gas chromatography and flame ionization detection. From the observed fiber concentration-effect relationship, Critical Fiber Residues (CFR) corresponding to 50% response in daphnia, algae and trout were determined. Fiber measurements performed for 38 petroleum substances including crude oils, fuels, hydrocarbon solvents and petrochemicals were then used to determine if CFRs derived from No. 2 Fuel Oil could be used to predict observed effects. Results indicate SPME biomimetic extraction correctly predicted the hazard classification for most of the products investigated but overstated observed aquatic toxicity in a few cases. Consequently, this technique provides a simple, conservative and cost-effective alternative test to support environmental classification of petroleum substances that avoids animal use.



Poster

The COLIPA strategy for the development of *in vitro* alternatives: Genotoxicity

Stefan Pfuhrer¹, Marilyn J. Aardema², Norbert K. Banduhn³, Rolf Fautz⁴, Jean-Roch Meunier⁵, Irene Manou⁶ and Alison Wolfreys⁷

¹Wella AG, Darmstadt, Germany; ²Procter and Gamble, Cincinnati, USA; ³Henkel, Duesseldorf, Germany; ⁴Kao Professional Salon Services, Darmstadt, Germany; ⁵L'Oréal, Paris, France; ⁶COLIPA, Brussels, Belgium; ⁷Unilever, Bedford, UK

The basic *in vitro* genotoxicity tests like the Ames test, mouse lymphoma test, chromosomal aberration or micronucleus test, exhibit major limitations such as the lack of human-like metabolic capacity, toxicokinetics, use of cell lines that are not relevant to the target organs and oversensitivity compared to *in vivo* situations. A recent analysis of over 700 chemicals tested in the current *in vitro* genotoxicity tests demonstrated that, whilst they are efficient at detecting rodent carcinogens, 75%-95% of rodent non-carcinogens also induce false positive results in one or more of these assays (Kirkland et al., *Mut. Res.*, in press) which leads to most chemicals requiring *in vivo* animal tests. Acknowledging these limitations of the present *in vitro* assays, a task force initiated by the European Commission and led by

ECVAM recommended the introduction of an additional *in vitro* step using skin models (Maurici et al., *ATLA*, in press). Taking on this recommendation, the COLIPA Task Force Genotoxicity has developed a concept for dermally exposed substances that should form part of a strategy for replacement of animal experiments. A preliminary work plan based on this concept will be presented. This aims at clarifying positive results from *in vitro* genotoxicity tests on the basis of *in vitro* experiments that adequately cover skin metabolism, skin penetration and genotoxicity. Ideally, the development and future validation of these methods will not only lead to replacement of animal experiments but also to the generation of results with higher significance for the dermal route of exposure.

Poster

Evaluation of U937 cell line for the identification of contact allergens

François Python¹, Pierre Aeby² and Carsten Goebel³

¹Cosmital SA (Wella AG), Toxicology, supported by The European Cosmetic Toiletry and Perfumery Association (COLIPA), Marly, Switzerland; ²Cosmital SA (Wella AG), Toxicology, Marly, Switzerland; ³Wella AG, Department of Product Safety, Toxicology, Darmstadt, Germany

The development of *in vitro* testing allows to reduce animal testing and to better analyse the complex mechanisms underlying dermal sensitisation. Having a central role in the initiation of allergic contact hypersensitivity, dendritic cells may represent an alternative to animal tests. Recently, our group and others reported promising results obtained with human peripheral blood monocytes derived dendritic-like cells (PBMDs). However, PBMDs major drawbacks are the complex and expensive method for obtaining them and donor-to-donor variability. To solve these problems, the U937 cell line was evaluated as a source of dendritic-like cells. Thus, we have developed an *in vitro* test for the identification of contact allergens based on the activation of dendritic-like cells. Cultured in presence of low concentration of interleukin-4 (IL-4), U937 cells were seeded in

12-well plates and exposed to test items for 24 hours, 48 hours and 72 hours. Cells were then analysed by flow cytometric measurement of the co-stimulatory molecule CD86 and by quantitative real time Reverse Transcriptase-Polymerase Chain Reaction analysis of IL-1 β and IL-8 gene expressions. Standard sensitizers, standard irritants and oxidative hair dye precursors were tested at non-toxic to sub-toxic concentrations. For each test item, specific modulation of the chosen markers (CD86, IL-1 β and IL-8) was observed, indicating that the described *in vitro* assay may be able to discriminate contact allergens from irritants. Five oxidative hair dye precursors were identified as potential sensitizers, confirming the results obtained with the murine local lymph node assay. A classification scheme based on the *in vitro* results is proposed.

**Poster****Primate and mouse Precision Cut Lung Slices (PCLS) as alternative for *in vivo* respiratory toxicology testing**

Katherina Sewald¹, Maja Henjakovic¹, Stefan Uhlig², Andrea Wohlsen³, Norbert Krug¹ and Armin Braun¹

¹Fraunhofer ITEM, Immunology and Allergology, Hannover, Germany; ²Research Center Borstel, Pulmonary Pharmacology, Borstel, Germany; ³Altana Pharma, Konstanz, Germany

Precision cut lung slices (PCLS) offer the distinctive opportunity to gain insight into lung functions under cell culture conditions. PCLS possess all the advantages of an *in vitro* technique but still maintain many functions of the intact organ. The objective of our work is the development of measurements of different proteomic and genomic endpoints for PCLS for rapid *in vitro* assessment and prediction of the respiratory sensitising/toxicological potencies of (bio)pharmaka and air pollutants.

Therefore, lungs of different species are taken to be filled with a medium-agarose solution and cut with a microtome (Krumdieck tissue slicer). After preparation of PCLS with a thickness of 250 µm slices are washed and cultivated for few days at 37°C. Vitality of the lung slices is remained for over 48 hours as controlled by LDH measurements and propidium iodide staining.

Here, we report the effects of direct exposure of primate or mouse lung slices to increasing concentrations of immunostimulants e.g. lipopolysaccharides (LPS), MALP-2 and TNFα. We show the quantification of cytokine generation assayed by flow cytometry on beads (IL1α, IL2, IL4, IL5, IL6, IL10, IL17, TNFα, GM-CSF, IFNγ) and ELISA (IL6, IL8, IL12, TNFα). Further studies using allergens like trimellitic anhydride (TMA), dinitrochlorobenzene (DNCB), amylase or detergent proteases and immune suppressive dexamethasone are planned for the near future. Beside this, gene microarray experiments of differentially expressed genes in the monkey model will be performed.

Poster**Improving drug product development and outcome with validated target organ specific human cell based three dimensional *in vitro* models**

John Sheasgreen, Mitchell Klausner* and Patrick Hayden

MatTek Corporation, R&D, Ashland, USA

A recent US Food and Drug Administration report "Innovation Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products" (March 2004) concludes that "traditional tools used to assess product safety have changed little and have not benefited from gains in scientific knowledge." Advances in harvesting normal human cells, optimisation of proliferation and differentiation media, and the application of the principles of GMP manufacturing have allowed MatTek to produce various target organ specific *in vitro* models which obviate or dramatically reduce the need for animals for a number of test methods. These models are based on normal human cells and thus avoid problems associated with species extrapolation from animal systems, an important consideration given the increasing reliance on genomic and proteomic studies. Careful

control of the culture system allows production of highly reproducible tissues which exhibit dramatically improved reproducibility versus animals or explants tissues. The expression of highly differentiated function within these tissues makes them suitable for a number of investigations involving tissue function, gene expression, permeability, and other differentiated phenomena for which simpler, monolayer cell systems are not well suited. In addition, in a number of instances, the *in vitro* tissue models are preferable to whole organism testing since the isolation of specific phenomena are possible allowing easier, more accurate, and reproducible test results. Finally second generation tissues that incorporate multiple cell types allow researchers to determine the contribution of the various cell types to the phenomena under study.



Poster

A novel bioreactor for *ex vivo* testing of biologic heart valves

Ulrich Stock¹, Gerhard Sammler², Katja Schenke-Layland³, Gerhard Ditze², Manfred Pohl⁴, Max Wendt⁴, Ralf Kühnle¹, Martin Hartrumpf¹ and Johannes Albes¹

¹Heart Center Brandenburg, Cardiovascular Surgery, Bernau, Germany; ²Friedrich-Schiller-University, Central Engineering, Jena, Germany; ³Saban Research Institute, Cardiovascular Surgery Research, Los Angeles, USA; ⁴Charite University, Medical Physics and Biophysics, Berlin, Germany

Introduction: Biologic heart valves substitutes are currently tested in large animals. The objective of this study was to design a bioreactor to simulate mammalian heart rate, blood pressure and flow in order to reduce animal experiments.

Methods: The entire hydrodynamic pulse replicator is assembled of medical graded materials to be easily and repeatedly autoclaved. The system consists of a cylindrical culture reservoir made out of borosilicate glass. A polycarbonate disc to house the heart valves is attached to a steel tripod. This tripod passes a steel lid and is connected to a pressure piston with a return spring. The steel lid covers the reservoir airtight allowing a prepressurisation with compressed air-carbon-dioxide-mix to maintain a physiologic oxygen and carbon dioxide content. The housing disc with the heart valves is pushed down in the culture reservoir using an attached pressure line and moves backwards

using the return spring. The application and release of pressure is microprocessor controlled. There are no heat-radiating components. The entire bioreactor can be placed in a conventional incubator.

Results: The microprocessor controlled movement of the housing disc with the heart valves results in an cardiac like cycle by means of complete opening and competent closing of the leaflets. The pulse rate can be adjusted up to 2 Hertz. Pressure recordings revealed a dirotic pressure profile resembling a physiologic pressure environment.

Conclusion: The simple, basically maintenance free, bioreactor design allows easy and reproducible simulation of a physiologic heart cycle. This is the first step to conduct feasibility studies of new biologic heart valves *ex vivo*, reducing currently required large animal experiments.

Poster

Evaluation of neuroactive compounds with neuronal networks *in vitro* on microelectrode neuro-sensor chips

Simone Stüwe¹, Alexandra Gramowski¹, Olaf Schröder², Liane Mehnert¹, Konstantin Jügelt¹, Elke Thedinga³ and Dieter G. Weiss¹

¹University of Rostock, Institute of Biological Sciences, Cell Biology and Biosystems Technology, Rostock, Germany; ²Pattern Expert, Borsdorf, Germany; ³Bionas GmbH, Rostock, Germany

Nerve cells growing directly on microelectrode neurochips organise into networks and communicate via chemical and electrical signals like *in vivo*. They generate typical signal patterns of electrical activity which can be recorded and analysed. The patterns are stable, reproducible, receptor- and tissue specific. Neuroactive compounds modify network activity in a substance-specific manner. By refinement of data analysis methods and machine learning approaches the vast amount of complex data can be assigned to substance specific profiles.

We compared several anticonvulsants and anaesthetics with varying modes of action with respect to their typical changes of electrical network activity patterns in cultures of murine frontal cortex. Simultaneous extra-cellular multielectrode recordings

reveal a concentration-dependent decrease of activity for all test compounds. Besides a general activity decrease (compound-specific effective concentration values), different compounds show distinguishable special effects on pattern synchronicity, oscillatory behaviour and intra-burst structure as well as reversibility.

Neuronal networks on microelectrode arrays have developed into a valuable technology that yields highly detailed insights into mechanisms of action and side effects of neuroactive drugs and prevents animal trials at an early stage of drug development.

Supported by Landesforschungsschwerpunkt Mecklenburg-Vorpommern Biosystemtechnik and European Community (ERDF).

**Poster****Establishment of human tissue banks for bio-medical research in Switzerland**

Massimo Tettamanti¹, Max Molteni*² and Shiranee Pereira³

¹ International Center For Alternatives In Research and Education (I-CARE), Chennai, India; ² ATRA, Lugano, Switzerland;

³ CIBA, Chennai, India

Introduction: This project has been propounded by ATRA and I-CARE with the purpose of establishing in Switzerland one or more human tissue banks for the sole purpose of bio-medical research. These human tissue banks will work to source, treat, preserve and supply human tissue material for research purposes.

Methods: Along with the establishment of human tissue banks the project will also emphasise the need to set up regulations to protect surgeons and researchers, in making clear that the use of human tissue for research purposes must never come in conflict with organ donation for transplantation and in creating a centre that can co-ordinate and catalogue all tissue suppliers and users.

Results: With this one project, it would be possible 1. To reduce the use of animals for experimentation in Switzerland by

15-20%, thereby saving up to 100.000 animals annually 2. To improve the quality of biomedical research 3. To improve the collaboration between specialists/surgeons and researchers who work in the laboratory.

Discussion: A large number of experiments in biomedical research are carried out on tissues of animal origin. More-meaningful results can be obtained by using human material in pre-clinical studies. It has been ascertained that the demand in Europe for human tissue by researchers is far greater than the supply. Surplus surgical tissue is considered a sanitary waste, but could be sourced and used as per national regulations. A human tissue bank would actually turn what is now considered waste into a resource for biomedical research.

Poster***Daphnia magna* as alternative bio-object in ecotoxicology**

Valeerii Tonkopii

Institute of Limnology, St. Petersburg, Russia

We have been developing non-traditional methods of the identification of pollutants, using various hydrobionts as biological objects and the study of the mechanism of toxic action of xenobiotics. The experiments were carried out with using of *Daphnia magna*. The toxicity of xenobiotics was determined by the value of LC₅₀, a concentration of the compounds causing death to 50% of hydrobionts during incubation with toxicants for 24 hours. The toxicity of organophosphates (Dipterex, DFP, DDVP, Paraoxon, Malathion, Malaaxon), heavy metals ions (Hg, Pb, Cu, Co, Cd, Cr, As, Al), organochlorines (Aldrin, Dieldrin, Endrin, Aroclor, DDT, Lindane, PCBs etc.), cyanides (sodium cyanide) and pyrethroids (Cypermethrin, Fenvalerate, Deltamethrin, Permethrin, Allethrin, Resmethrin, Phenothrin, Kadethrin, Cyphenothrin) was determined. The effects of a number of antagonists on the toxicity of xenobiotics were stud-

ied. At the first time we discovered that in experiments to *Daphnia magna* some muscarinic cholinoreceptor blockers (atropine, amyzil etc.) reduced the toxic effect of organophosphates. In the case of heavy metals the chelating agents (EDTA, Dithioethylcarbamate, Unithiolium, Sodium thiosulphuricum, L-Aspartic acid) were effective, for certain organochlorine poisonings – anticonvulsive drugs (diazepam, phenobarbital), for cyanide poisoning – sodium nitrite and anticyane. In the case of pyrethroid's poisonings the antagonist of glutamate receptor (ketamine) and agonists of GABA-receptor (phenazepam, ethanol) reduced the toxicity of xenobiotics. As far as these antidotes have a specific treatment action only against definite classes of pollutants, we have elaborated the sensitive express-methods of bio-identification of pollutants with the usage of alternative bio-object.



Poster

Precision-cut fibrotic liver slices as a new *in vitro* model to study fibrosis and to test anti-fibrotic drugs

Marja van de Bovenkamp, Geny Groothuis and Peter Olinga

University of Groningen, Department of Pharmacokinetics and Drug Delivery, Groningen, The Netherlands

Introduction: Liver fibrosis is one of the leading causes of death worldwide. Studies on anti-fibrotic drugs rely mostly on *in vivo* models involving substantial animal discomfort. Current *in vitro* models cannot mimic the complex *in vivo* milieu. Therefore, we evaluated fibrotic liver slices as a new, physiologic *in vitro* model to study fibrosis and to test anti-fibrotic compounds.

Methods: Precision-cut liver slices (8 mm diameter, 250 μ m thickness) were prepared from livers of rats three weeks after bile-duct ligation (BDL) and incubated for 0-48 hours with 0-2 mM pentoxifylline. Expression of fibrosis-markers was studied using real-time PCR, Western blot, and histochemistry. Viability was assessed by measuring ATP content.

Results: BDL-livers showed clear signs of fibrosis, like increased collagen content and α -smooth-muscle actin (α SMA) expression. Fibrotic liver slices remained viable during 48 hours

of incubation with significant increase of pro-collagen-1 α 1 mRNA expression and collagen protein content (5.7 ± 0.7 and 1.5 ± 0.07 fold compared to non-incubated slices), indicating progression of fibrosis. Addition of the anti-fibrotic drug pentoxifylline inhibited pro-collagen-1 α 1 mRNA, α SMA mRNA and α SMA protein expression significantly after 24 hours of incubation (0.26 ± 0.04 , 0.22 ± 0.13 and 0.82 ± 0.02 fold compared to control incubation) without influencing slice viability.

Conclusion: Fibrotic liver slices are a promising tool to study fibrosis *in vitro* in a physiological, multicellular context and to test anti-fibrotic drugs. Importantly, this method may provide the opportunity to study these processes not only in animal, but also in fibrotic human liver tissue and will contribute substantially to the reduction, refinement, and potential replacement of animal experiments.

Poster

Differential susceptibility of lung cell lines to cytotoxic effects of respiratory irritants and sensitisers

Sandra Verstraelen¹, Rosette Van Den Heuvel¹, Inge Nelissen¹, Geert Verheyen¹, Paul Van Cauwenberge² and Greet Schoeters¹

¹Flemish Institute for Technological Research, Centre of Expertise in Environmental Toxicology, Mol, Belgium;

²Ghent University, Department Otorhinolaryngology, Ghent, Belgium

Occupational exposure to chemicals is one of the main causes of respiratory allergy and asthma in Western countries. Identification of chemicals that trigger asthma is difficult due to the lack of a validated *in vitro* test method. In our approach to create an *in vitro* assay for respiratory toxicity, the choice of a relevant cell model is important. Different human cell models modelling for different structures of the upper and lower airways may demonstrate variable susceptibility to cytotoxicity caused by model respiratory sensitisers and irritants. The objective of the current work was to compare the susceptibility of selected cell lines and examine whether this susceptibility correlates with the *in vivo* localisation of the represented lung structure and its accessibility to inhaled chemicals.

The human bronchial epithelial cell line Beas-2B, the alveolar epithelial cell line A549 and macrophages derived from the

myelomonocytic cell line THP-1 were exposed to the respiratory sensitising chemical ammonium hexachloroplatinate IV and the irritant tributyltin. After 24 hours cytotoxicity was measured by a neutral red uptake assay.

The cytotoxic effects of the chemical ammonium hexachloroplatinate IV according to EC50 values could be ranked as follows: Beas-2B (most sensitive) < THP-1 < A549 (least sensitive). Similar observations were made after exposure to the respiratory irritant tributyltin.

Our observations suggest that human cell lines modelling for different lung structures show a differential susceptibility to respiratory sensitising and irritating chemicals which correlates with their ability to physically contact inhaled chemicals *in vivo*.



Lecture

NeuroSensorix[®]: CMOS neurochip for long-term recording from neuronal networks *in vitro*

Dieter G. Weiss¹, Werner Baumann¹, Erik Schreiber¹, Guido Krause¹, Angela Podssun¹, Mirko Lehmann², Ingo Freund², Simone Stüwe¹, Alexandra Gramowski¹, Konstantin Jügelt¹, Olaf Schröder³ and Ralf Ehret⁴

¹University of Rostock, Rostock, Germany; ²Micronas GmbH, Freiburg, Germany;

³Pattern Expert, Borsdorf, Germany; ⁴Bionas GmbH, Rostock, Germany

Neuronal tissue and a suitable recording/stimulating electronic system form a functional bio-electronic hybrid system. This system provides a novel *in vitro* platform for pharmaceutical drug development, for high-content drug screening, and for safety pharmacology.

We culture electrically active neuronal networks from embryonic mouse spinal cord or brain directly on glass/ITO- or silicon-based multi-electrode arrays with stable cell-electrode coupling for several months. This allows the monitoring of the onset of electrical activity, of bursting activity stabilisation and of the development of histotypic native or drug-modified electrical activity patterns. The glass neurochip sensor system was extensively used over the last years to monitor states of toxic or metabolic impairment of neurons accompanied by characteristic electrical activity changes. Results will be reported from studies on the effects on the

electrical activity of neurotoxins, ammonia, neurosteroids, benzodiazepines, anaesthetics and anticonvulsive drugs as well as studies on detecting neuronal side-effects of compounds.

In addition, a new standard CMOS technology-based silicon chip with unique features has recently been introduced. Besides the recording electrodes for action potentials, temperature diodes and ion sensitive field effect transistors (ISFET) were integrated to measure temperature and pH changes and oxygen concentration of the cultures at the silicon chip.

This NeuroSensorix[®] approach reduces the number of animal experiments, refines the quality of data analysis and will replace animal tests.

Supported by the State of Mecklenburg-Vorpommern and European Community (ERDF).

Poster

Influence of hypothyroidism induced by thiamazole on the toxic interaction between propranolol and disopyramide in chick embryos

Yuji Yoshiyama and Motoko Kanke

Kyoritsu University of Pharmacy, Division of Clinical Pharmacy, Tokyo, Japan

In order to develop alternative methods, we have studied the biological effects of drugs on the cardiovascular system of chick embryos using physiological techniques. The present study evaluated the effect of the hypothyroidism induced by thiamazole on the toxic interaction between propranolol and disopyramide in chick embryos.

Fertilised eggs of White Leghorns were incubated and investigated. 1.2 mg/0.2 ml/egg of thiamazole was injected into the albumen of fertilised eggs of incubation. The control group was given 0.2 ml/egg of physiological saline in the same manner. Propranolol at 0.1 mg/egg and disopyramide at 0.3 mg/egg were injected into the air sac of fertilised eggs of incubation. Electrocardiograms (ECGs) were recorded 0 to 60 min after the injection.

Results: After the injection of propranolol and disopyramide into the thiamazole treated eggs, the heart rate was significantly

decreased compared with the thiamazole untreated eggs. In addition, this toxic interaction between propranolol and disopyramide was more severe at the chick embryos with hypothyroidism induced by thiamazole.

Discussion: An experimental animal model with heart disease originated from abnormalities of the thyroid gland in chick embryos has been produced by the treatment with thiamazole. With the recent concern for animal rights, experimental studies using mammals have been limited. In the present study, the influence of the hypothyroidism on the toxic interaction between propranolol and disopyramide was demonstrated in chick embryos. In conclusion, the chick embryonic model of hypothyroidism produced by thiamazole may be useful for investigating the pharmacological and toxicological effects of cardiovascular drugs.