Session 7.4
Non-invasive techniques for monitoring and imaging (Doerenkamp-Zbinden-session)

Lecture

Magnetic Resonance Imaging (MRI) of the lung as a tool for the non-invasive evaluation of drugs in rat models of airways diseases

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The inflammatory status of the lungs in rat models of airways diseases is traditionally inferred from Bronchoalveolar Lavage Fluid (BALF) analysis and/or histology. For lung function analysis rats are usually tracheotomised and artificially ventilated. To suppress spontaneous respiration animals receive a muscle relaxant. From measurements of airflow and transpulmonary pressure, airway resistance is calculated after each respiratory cycle. Clearly, the invasive character of these procedures precludes repeated assessments in the same animal.

We have developed MRI approaches in which acquisitions are performed on spontaneously breathing, anaesthetised animals. Neither artificial ventilation nor tracheotomy is necessary. Therefore, interference with the pathophysiology and discomfort for the animals should be minimal. In addition, repetitive measurements are feasible. We estimate that this leads to a reduction in the number of animals used for this sort of experiments by at least 70%.

Two illustrative examples are allergen (ovalbumin, OVA) and lipopolysaccharide (LPS) inducing distinct inflammatory responses in rats. For OVA, MRI signals correlate with eosinophilia and increased protein content in BALF. Following LPS, MRI signals reflect secreted mucus as revealed in BALF. Histology confirms these results.

The effects of airways remodelling and hyporesponsiveness induced by respectively OVA or LPS can also be monitored by detecting modulations of the parenchymal signal caused by changes in oxygenation levels. This opens the avenue for non-invasive assessments of lung function.

Overall, these approaches provide the basis for non-invasive testing of anti-inflammatory compounds for respiratory diseases.

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Poster

Analysis by FIB imaging technique of Caco-2 cell lines cultured on a new tridimensional substrate

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Epithelial cells cultured in a tridimensional (3D) environment could improve their growth and differentiation, offering an *in vitro* model closer to *in vivo* situation. Human intestinal Caco-2 cells, able to spontaneously differentiate in long-term culture to small enterocytes and extensively used as model of intestinal barrier, are particularly suitable and intriguing for this advanced culture technique.

Biopolymers have been demonstrated to be an efficient support for epithelial cells; in particular alginate, an anionic mucus-adhesive polymer produced from different species of brown algae, has several unique properties of entrapment and/or delivery of the cells.

In this study, we have investigated growth capability and morphology of parental Caco-2 cells and Caco-2/TC7 clone, on a new 3D alginate concave encapsulation model. In this system, both cell lines actively grow, increasing their number (till to 500%) for about 15 days and remaining viable until the 21 day of culture, as in monolayer condition.

Our 3D culture model has been analysed by Focused Ion Beam-Scanning Electron Microscope (FIB/SEM), an innovative imaging and manipulation technique for biological samples; it allows selective sectioning and imaging at nanoscale, collecting secondary particles generated by primary ions or electrons. The technique proved viable for investigation of the boundary between cells and host matrix.

Even if further functional studies must be conducted, present results show that alginate matrix is able to support cellular growth and morphological features of Caco-2 and Caco-2/TC7 cells, opening new perspectives for a more physiological organisation in culture of these cells.

Poster

Biophotonic imaging and its uses for monitoring and tracking disease processes in live animals

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Xenogen Corporation is a leader in the field of biophotonic imaging and has developed a technology which allows biological processes, including gene expression that is both temporal and spatially defined (i.e. occurring in defined tissues and organs), to be monitored in live animals in real-time. Genes encoding luciferase and fluorescent proteins are engineered into cells (e.g. cancer cell lines and infectious disease agents) and animals (transgenic mice and rats) to enable them to produce light that can be visualised through the tissues of a live animal using specialised imaging equipment and software designed and built by the company. To date, Xenogen's technology has been used predominantly to facilitate research in areas such as oncology, infectious disease, inflammation, neurology and toxicology. This non-invasive imaging technique allows significantly fewer animals to be used due to the generation of superior data and better biostatistics. An overview of this technology will be presented along with specific examples in each of the above disease areas.

Lecture

Measuring nociception by fMRI in anaesthetised animals

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There is a demand for novel analgesics to provide relief for different types of pain. The development of new analgesics depends on traditional pain tests in animals. They measure behavioural reactions of awake animals suffering from pain. A solution could come from measuring responses to painful stimuli in the anaesthetised animal non-invasively by functional Magnetic Resonance Imaging (fMRI). This method provides highly resolved objective functional information on the processing of nociceptive stimuli throughout the whole brain as has been demonstrated in human pain studies. Consequently, this method could also improve the objective measurement of modulatory effects of analgesics.

We established such a fMRI testing system in anaesthetised rats using a mild noxious heat stimulation applied to the rat hindpaw. Because the testing is applied to animals under anaesthesia and the stimulation is mild (temperature: 34-45°C, suitable for humans too) we are minimising the stress for the animal. Moreover, we obtain reliable objective information of different pain competent structures along the pain pathway throughout the whole brain. Having established an optimised analytical framework we could define the degree of pain suppression of different conventional analgesics. Moreover, such a model can be applied for investigating (chronic) pain processes. This would open a new avenue for research on pain chronification and it may contribute to the evaluation of novel analgesics intended to inhibit or even reverse chronic pain.

Poster

Non-invasive imaging of pulmonary tumours in mice using Flat-Panel Detector-based Volumetric Computed Tomography

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There is a strong need for non-invasive methods to monitor tumour growth and progression over time. This study was aimed at examining the usefulness of Flat-Panel Detector-based cone beam Volume-Computed Tomography (FPD-VCT) as a non-invasive technique to monitor tumour growth and metastatic spread at defined time intervals.

1x106 A549 cells (human lung adenocarcinoma cell line) were injected through the intercostal muscular tissue directly into the left lower lobe of the lung of adult SCID mice (n=8). Two weeks after tumour cell inoculation, scans of anaesthetised mice were performed using FPD-VCT (General Electrics Prototype) and were repeated at distinct time intervals.

The results obtained by FPD-VCT were subsequently related to histological analyses. Pulmonary tumour nodules of about 500 µm in diameter could reproducibly be detected by FPD-VCT. Monitoring of tumour growth pattern, morphological characteristics, perfusion rate and tumour extension by FPD-VCT allows a continuous view inside the animal and assessment of the course of the disease. Furthermore this imaging technology might be a powerful tool to reduce animal pain and distress, by assessing the tumour load of the animals. It will also allow for a significant reduction of the number of animals needed to sacrifice for experimental purposes, because imaging can be repeated as often as necessary.

Thus, FPD-VCT analysis has the potential to establish a novel standard for real-time non-invasive tumour assessment over time in animal models.

Poster

Multi-parametric digital imaging test battery for optical monitoring of the physiology of living cells *in vitro*

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A multi-parametric live cell imaging approach with state-of-the-art high resolution video-enhanced, confocal, DIC, POL and digital fluorescence microscopy is used as test battery for the monitoring of physiological reactions to the addition of test compounds. Application to neuronal network cultures allowed a distinction of the different glial and neuronal cell types, their axonal and dendritic processes and a direct comparison of the pharmacological impairment of electrical activity with Ca2+ level oscillations. Further studies to compare intracellular activity such as organelle movement, cytoskeleton restructuring, or Ca2+ signals with electrical activity in a physiologically active neuronal cell ensemble are in progress. The system allows highly complex studies of cellular behaviour which give new insight into the molecular mechanisms of drug action. Since multiple endpoints can be determined, this test battery will be especially useful in studies where toxic or adverse side effects of test compounds need to be excluded, because its multi-parametric feature reduces the extent of false negative results considerably.

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Poster

Imaging induced neurodegeneration in living fluorescent cells using organotypic hippocampal slice cultures derived from transgenic fluorescent mice

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Transgenic mice expressing fluorescent proteins in neurons and glia provide new tools for on-line visualisation of dynamic degenerative and regenerative structural changes. In initial trials we have established hippocampal slice cultures derived from 7 day old transgenic fluorescent mice of several strains. With thy1 as promoter, one strain expresses EYFP (Enhanced Yellow Fluorescent Protein) in subpopulations of pyramidal cells and processes; a second expresses CFP (Cyan Fluorescent Protein) in the same subpopulations, as well as in a subpopulation of dentate granule cells and their mossy fibres. A third strain expresses DsRed in all astrocytes under the GFAP promoter. Combining the last two strains has provided double transgenic pups with red glial cells and cyan neurons expressed in hippocampal slice cultures. Most recently we have characterised hippocampal slice cultures from PLP-GFP (Green Fluorescent Protein) mice expressing fluorescence in oligodendrocytes. Here we report the presence and developmental expression of the transgene proteins allowing visualisation of neuronal and glial cell bodies and processes in hippocampal slice cultures, as well as the disappearance of fluorescence in relation to experimental excitotoxic and mechanical lesions. Fluorescence was recorded before and then followed after the lesion induction by time-lapse fluorescence microscopy. The method is useful for screening of compounds for neurotoxicity as well as glial toxicity. Examples, including time-lapse videos, will be presented at the meeting.

Trangenic mice were kindly provided by Prof. Aagaard Jensen, MBC and Dr. Zalc, Inserm and obtained from Jackson Laboratories. The study was supported by the Danish MRC and the FP5 EU-grant (QLK3-CT-2001-00407).

Lecture

New approaches in non-invasive molecular imaging: Combining PET and MRI

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Positron Emission Tomography (PET) provides quantitative information about functional processes such as glucose metabolism, receptor-ligand binding, and even gene expression. Its high sensitivity in the picomolar range and the large variety of radiolabelled tracers and markers makes PET a powerful tool for functional *in vivo* molecular imaging. Since PET is absolutely non-invasive, the number of used animals for longitudinal follow-up studies can be drastically reduced and the revealed data become more reliable since the same animal is used over the entire period. However, PET studies show only very little anatomical information and especially for new tracers and biomarkers with an unknown biodistribution, it is often hard to determine their uptake localisation *in vivo*. Many efforts are made to combine high resolution PET with Computed X-Ray Tomography (CT), an imaging modality providing advanced information about the anatomy. However, CT has low soft tissue contrast and uses relatively high doses of ionising radiation, which might have biological effects in the animal models being studied. As an alternative, Magnetic Resonance Tomography (MRT) can provide high spatial resolution and excellent soft tissue contrast for morphological imaging, but suffers from poor signal strength leading to low sensitivity for functional imaging. Thus, it would be ideal to combine PET and MRI in one device and derive functional and anatomical information at the same time. Our groups focus on the development of a combined PET-MRI scanner for small animals based on novel avalanche photodiodes. First studies proved the feasibility of such a combined system.

Lecture

In vivo imaging in drug discovery and development

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Drug discovery and development is a long-lasting process (on average 15 years) involving many different disciplines and producing a lot of costs up to 1.2 billion US$/compound until launched to the market. Recently the FDA stated solicitously an ongoing tendency of increasing costs for drug development which might lead to an unbearable burden for the health care system. Therefore the FDA started several initiatives to involve more strongly new promising technologies to accelerate this process to reduce costs and to increase success rates for compounds.

In vivo imaging, especially Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) became important tools to improve that process. With imaging, we can perform longitudinal studies on single animals without the sampling error inherent to biopsy; however, the most important attribute of imaging is the provision of structural and functional information under physiologic conditions, mimicking the situation observed in the clinic. Disease is a biological process caused by changes at the molecular level, therefore molecular imaging, mostly using PET together with tracer amounts of radio-actively labelled compounds, can hasten drug development at the target identification and validation stages, in the synthesis and optimisation of drug candidates, and in pre-phase I to phase II clinical trials, i.e., at almost any point in the process. Furthermore the translational aspect of both technologies to look at the same readout in animal as in man helps to improve the predictability of animal models, to reduce the number of animals needed and to decrease development time for drugs.

Poster

Online monitoring of physiological parameters of cell cultures

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Physiology parameters such as oxygen consumption and acidification showed to be important parameters in cell metabolism. Parameter changes in dependence of test compounds or supplements are meaningful indicators for metabolic interferences like receptor activation, initiation of signal transduction pathways, irritation/corrosion, inhibition or activation of metabolic pathways and apoptosis.

Therefore, we developed our "BIONAS®2500 analysing system" suitable for online detection of long term effects on physiological parameters on living cells. Our system is able to monitor oxygen consumption, acidification and adhesion of living cell cultures. It is a useful tool for analysing cell reactions in dependent of test substances. The detection can by performed from a few hours to several days. Regeneration/recovery effects can also be monitored after displacement of the substance or supplement.

Another point of interest is the measurement of changes in cell adhesion and confluence. As above mentioned, it can also be measured by the "BIONAS®2500 analysing system". Especially for adhesion detection we developed a new Multiwell analysing system "BIONAS®9600 AdCon Reader". It allows monitoring of changes in cell morphology and cell shape, cell proliferation, cell adhesion and spreading and also cell death (apoptosis/necrosis). Furthermore receptor activation, signal transduction, irritation/corrosion and inhibition/activation of metabolic pathways can be detected. It can be also very useful for cell differentiation analysis and other applications.

Poster

The optical probe technique for the analysis of drug resistance profiles in multicellular tumour spheroids

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The development of multidrug resistance (MDR) is a major impediment for the success of chemotherapeutic cancer treatment. MDR mainly occurs due to increased expression of MDR transporters belonging to the ABC-family of transporters, e.g. P-Glycoprotein (Pgp), Multidrug Resistance-associated Protein-1 (MRP-1), and Breast Cancer Resistance Protein (BCRP). To circumvent MDR an increasing number of MDR-reversing agents have been developed and are tested either in two-dimensional cell cultures or in animal experiments of cancer. In a novel approach to replace animal experiments the optical probe was developed. This new technique uses the optical sectioning properties of confocal laser scanning microscopy to record drug penetration profiles in the depth of three-dimensional tumour tissues, i.e. either multicellular tumour spheroids or tumour fragment spheroids. Following staining with either fluorescent anti-cancer agents or fluorescent test substances drug penetration and diffusion kinetics of these probes can be monitored semi-automatically over time in living tissues. This allows routine testing of MDR reversing agents which increase the diffusion of anti-cancer agents in treated tissues, and is applicable for large throughput screening.