National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Overcoming the barriers to wider uptake of human tissue for safety assessment

15 July 2014

Pioneering Better Science

About the hosts

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is an independent scientific organisation. It supports the UK science base by driving and funding innovation and technological developments that replace or reduce the need for animals in research and testing, and lead to improvements in welfare where animals continue to be used. The Centre promotes robust and ethical scientific practice through collaborating with research funders, academia, industry, regulators and animal welfare organisations, both in the UK and internationally. The NC3Rs is supported primarily by Government, but also receives funding from the charitable and industrial sectors. It has an annual budget of approximately £7 million and is the UK's major funder of 3Rs research. Further information about NC3Rs activities and programmes can be found at www.nc3rs.org.uk.

The MHRA is responsible for regulating all medicines and medical devices in the UK by ensuring they work and are acceptably safe. Underpinning their work lies robust and fact-based judgments to ensure that the benefits justify any risks. The MHRA is a centre of the Medicines and Healthcare Products Regulatory Agency which also includes the National Institute for Biological Standards and Control (NIBSC), and the Clinical Practice Research Datalink (CPRD). The MHRA is an executive agency of the Department of Health. Further information about the MHRA can be found at www.mhra.gov.uk.

Agenda

09.00 – 09.30	**Registration and poster/exhibitor viewing**
09.30 – 09.40	**Welcome and introduction** *Frank Bonner (Chair), Stem Cells for Safer Medicines*
Human tissues for cardiovascular safety studies	
09.40 – 10.05	**Human vascular tissue in safety assessment** *Hugo Vargas, Amgen*
10.05 – 10.30	**An iPSC cardiomyocyte multi-electrode array model** *James Louttit, GlaxoSmithKline*
10.30 – 10.55	**Human stem cell research for cardiac safety: Janssen's on-going strategy with data from 60 reference compounds, 4 different cell providers assessing different technologies** *David Gallacher, Janssen Research & Development*
10.55 – 11.25	**Coffee and poster/exhibitor viewing**
Human tissues for respiratory safety studies	
11.25 – 11.50	**3D human airway tissues for safety assessment: overcoming the barriers in safety assessment** *Samuel Constant, Epithelix*
11.50 – 12.15	**Fresh, functional human tissues and the prediction of drug safety** *David Bunton, Biopta*
Regulatory and supply issues – real or perceived barriers to human tissue adoption?	
12.15 – 12.30	**A vision for funded collections of human tissue and biosamples** *Jon Fistein, Medical Research Council*
12.30 – 13.00	▪ David Jones – Medicines and Healthcare Products Regulatory Agency ▪ Abby Jacobs – Food and Drug Administration ▪ Markku Pasanen - External non-clinical expert (SWP, SAWP) for EMA
13.00 – 14.00	**Lunch and poster/exhibitor viewing**
Breakout group discussions	
14.00 – 14.10	**Introduction to the breakout groups**
14.10 – 16.15	**Breakout groups**
16.15 – 16.45	**Coffee and poster/exhibitor viewing**
16.45 – 17.15	**Actions, next steps and timelines** ▪ Feedback from the breakout groups and a strategy for next steps will be presented
17.15 – 18.30	**Meeting close; networking and poster/exhibitor viewing**

Human vascular tissue in safety assessment

Hugo Vargas, Amgen

Ex vivo analysis of animal vascular tissues have been a valuable tool in the safety pharmacology laboratory to profile the activity of new drugs, and assess both target and off-target effects. Utilization of human vascular tissue can add value to cardiovascular risk assessment, by expanding the pharmacological profile of a new candidate drug in the "most relevant species", which can facilitate the translation of pharmacological activity (and risk potential) from nonclinical species to humans. In addition, evaluation of the "right vascular bed" (e.g., coronary versus non-coronary arteries) can address specific safety concerns that might be attributed to drug treatment (e.g., potential for myocardial ischemia due to coronary artery contraction). This talk will highlight the pros/cons of using human vascular tissue segments (from donors) in the setting of cardiovascular safety pharmacology testing.

Biography

Hugo is a Scientific Director in the Department of Toxicology Science at Amgen since 2006, and serves as the Head of the Safety & Exploratory Pharmacology group. He is responsible for the cardiovascular safety evaluation of new drugs considered for preclinical development, and for regulatory safety pharmacology studies that enable first-in-man testing. His experience covers early stage screening to clinical issue resolution.

Hugo has a PhD in Pharmacology from the Rutgers Graduate School of Biomedical Science (1988) and completed a Post-doctoral Fellowship in Pharmacology at UCLA (1991). He is a past President of the Safety Pharmacology Society, and was recently recognized as a Diplomate of Safety Pharmacology for his contributions to the discipline.

Hugo entered the pharmaceutical industry in 1991, and worked at Aventis, DuPont Pharma, and Merck Research Laboratories prior to joining Amgen.

An iPSC cardiomyocyte multi-electrode array model

James Louttit, GlaxoSmithKline

Functional cardiotoxicity, notably QT prolongation and proarrhythmic risk, is a leading cause of drug attrition during development. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) with multi-electrode array (MEA) technology could be used for predictive electrophysiological cardiac safety screening. For wider acceptance of assays using these cells, hiPSC-CMs must be validated against existing preclinical cardiac safety assays to determine assay predictivity in relation to current standards. Validating a particular cell type and/or batch of cells with reference compounds is key to setting up a robust preclinical assay for interspecies comparison of cardiac safety endpoints. hiPSC-CMs studied on MEAs were pharmacologically validated using a set of reference compounds and results compared to other preclinical cardiac safety assays used to assess cardiovascular electrophysiological risk (patch clamping, rabbit ventricular wedge assay, rodent and non-rodent *in vivo* models). Our results show that hiPSC-CMs demonstrate relevant pharmacological responses and show good correlation to other preclinical assays. Based on these data, we believe hiPSC-CMs could provide an alternative *in vitro* model to screen for drug induced cardiovascular electrophysiological risk and reduce the number of animals currently used for *in vitro* studies. We will also discuss the barriers to use of this assay type in a functional cardiovascular risk identification strategy.

Biography

James has 30 years' experience in the pharmaceutical industry working as a scientist and programme leader in drug discovery and development in GSK, legacy companies and academia. Areas of work have included --- hypertension, arrhythmias, atherosclerosis, ischaemia/reperfusion injury and cardioprotection. In particular he has extensive expertise in developing and running *in vivo* and *in vitro* models of cardiovascular function and disease.

James joined Safety Pharmacology in Jan 2010 as a Senior Cardiovascular Investigator and became Head of Safety Pharmacology UK in mid-2012.

Human stem cell research for cardiac safety: Janssen's on-going strategy with data from 60 reference compounds, 4 different cell providers assessing different technologies

David Gallacher, AN Hermans, I Kopljar, J Rohrbacher and HR Lu

Janssen Research & Development

We investigated the effects of 20-60 reference compounds of different mechanisms of action, (*mostly known to have cardiac effects*) in 4 different types of hES-CMs and hiPS-CMs, and using different technologies. We also compared data of 20 well known reference compounds with the current standard action potential model - the *in vitro*-isolated rabbit wedge model. In addition, we investigated the cardiac ion channel gene expression from each of the providers of these hES-CMs and hiPS-CMs. Our data suggest that hiPS-CMs with High Throughput/High Content screening technologies (*Ca2+ transients and Optical AP*) could be suitable to detect drug-induced QT-prolongation, shortening, and increases in beat rate in early de-risking research in support of drug discovery projects.

Biography

David has a broad experience and interest in cardiovascular physiology and pharmacology, including the central brain structures involved in cardiorespiratory control. He has collectively more than 25 years' research experience from working in academic (University College London, UCL), a CRO (Quintiles, Scotland) and different pharmaceutical settings (Pfizer UK and Janssen R&D, Belgium). At Pfizer Central Research he was a member a discovery research team running various models of stroke disease. He later went to the Royal Free Hospital School of Medicine (UCL) in London to do his PhD. His PhD research looked into the role of the cerebellum in cardiorespiratory control in relation activation of defensive behaviour brain structures such as the amygdala, hypothalamus and peri-aqueductal grey. During his short postdoctoral period at UCL he conducted research into the neonatal development of the baroreceptor reflexes. In 1996 he joined a new company, Quintiles Scotland Ltd in Edinburgh (Ex-Syntex Research), where he started, developed and managed the *in vivo* cardiovascular research group providing discovery model support and safety pharmacology services. In Sept 2003 he was asked to take over management of the Center of Excellence for Cardiovascular Safety Research for Johnson & Johnson PRD and has been given increasing roles of responsibility for Janssen R&D (*previous J&J PRD*), including Mechanistic Pharmacology and more recently the integrated global leadership of both early de-risking non-GLP safety pharmacology and regulatory GLP research. His team of safety experts provide support from de-risking in discovery through clinical development, licensing and up to marketed product support.

3D human airway tissues for safety assessment: overcoming the barriers in safety assessment

Samuel Constant, Epithelix

In vitro assessment of inhalation toxicity is an emerging and fast growing field. Until now only animal models are used in OECD test guidelines for inhalation toxicity testing and therefore *in vitro* alternatives of human origin are urgently needed. In the present talk, we will present and discuss the applications of 3D cell model of the human airway epithelia for acute, long term and repeated dose testing. A special emphasis will be given to the necessary steps and hurdles that a model/method developer needs to overcome during the development, optimization, scale-up and eventual acceptance of the tissue models by the end-users as well as the legislators.

Biography

Samuel is a co-founder/ Chief Operating Officer of Epithelix (http://www.epithelix.com) and co-founder/Chief Executive Officer of OncoTheis (http://www.oncotheis.com) two Swiss biotech companies specialized in tissue engineering.

Epithelix is a leader for *in vitro* assessment of drug efficacy and toxicity on human respiratory tract. Epithelix has developed unique 3D *in vitro* human airway tissues and testing services for studying airway pathologies like Asthma, Cystic Fibrosis and Chronic Obstructive Pulmonary Diseases. Samuel is in charge of global management and business development of the company; he also deals with the external collaborations with private and public research groups and financial issues.

Within the past 8 years, Samuel and his team have focused their research efforts on the development of novel *in vitro* approaches to study effect of inhaled xenobiotics on the human respiratory tract. His efforts have led to the development of methods for repeated toxicity testing of xenobiotics (90 Days repeated dose exposure), evaluation of respiratory absorption and metabolism, assessment of lung inflammation. Since 2006, Samuel and his team have won 15 prizes for their scientific achievements, technological innovation, and business development.

Fresh, functional human tissues and the prediction of drug safety

David Bunton[1], Graeme Macluskie[1], Karen McDaid[1], Cristina I Linde[2] and Keith C Bowers[1]

[1] Biopta Ltd, UK

[2] Biopta Inc, US

Human functional tissues are increasingly being used to assess the safety of preclinical drug candidates. Human fresh tissues have long been considered the closest possible model of human pharmacology because they closely retain the tissue phenotype and can be used to measure a wide range of pharmacological responses. Moreover, there is considerable capacity to make better use of human tissues that are residual to surgery or transplant procedures: over 95% of patients are happy to donate surgical tissues to research[1] and there are over 650,000 surgical procedures in England and Wales each year[2].

Despite this, relatively little drug development is conducted using fresh human tissue because of the perceived logistical and ethical difficulties surrounding the availability of tissue and practicalities of experimental work. Overcoming the barriers to uptake of human tissue research remains a challenge and will be supported by clear evidence of the benefits of such an approach.

Evidence is presented from examples where preclinical human tissue assays have successfully predicted clinical adverse effects and/or where there are accepted shortcomings in existing models[3,4,5] for example, *in vitro* measures of bronchoconstriction, the vascular component of blood pressure regulation, effects on cardiac contractility, or measurements of gastrointestinal motility or fluid secretion. Such studies resemble small clinical trials: patients are 'recruited' by defining the acceptance criteria for donated tissue specimens and comparisons can then be made between different patient groups, allowing a preclinical safety study to be conducted. It is also important to point out that such assays do not by themselves completely replace existing safety tests but contribute to a platform of evidence that increases the probability of clinical success and reduces the risk that species differences will go undetected.

References

1. Ownership and uses of human tissue: what are the opinions of surgical in-patients?. J Clin Pathol. 2008; 61: 322–326. Bryant, R.J., Harrison, R.F., Start, R.D. et al.
2. Defined as procedures involving "excisions or partial excisions" Hospital Episode Statistics, Admitted Patient Care 2011-12 in England.
3. Comparison of the Intrinsic Vasorelaxant and Inotropic Effects of the Antiarrhythmic Agents Vernakalant and Flecainide in Human Isolated Vascular and Cardiac Tissues. J Cardiovasc Pharmacol 2013;61:226–232. Lynch J.J., Regan C.P., Gregory N., Beatch G.N., Gleim, G.W. & Morabito, C.J.
4. The inability of tegaserod to affect platelet aggregation and coronary artery tone at supratherapeutic concentrations. Naunyn-Schmiedeberg's Arch Pharmacol (2012) 385:103–109 Higgins L. et al.
5. Discovery of N-(3(1-Methyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-indol-6-yl) thiopene-2-carboximidamideas a Selective Inhibitor of Human Neuronal Nitric Oxide Synthase (nNOS) for the Treatment of Pain. Journal of Medicinal Chemistry 20: 7408:7416 (2011). Annedi S.C., Maddaford S.P., Mladenova G., Ramnauth J., Rakhit S., Andrews J.S., Lee D.K.H., Zhang D., Porreca F., Bunton D.C., Christie L.

Biography

David is co-founder and CEO of Biopta Ltd. After completing a PhD in vascular biology, he accepted a Lectureship in Physiology and Pharmacology at Glasgow Caledonian University, where he commenced an intensive period of research into the development of methodologies for the testing of new medicines, culminating in the spin-out of Biopta in 2002 and the launch of its PM-1 tissue testing instrument. Biopta has since grown to employ 20 people and in 2012 opened a US subsidiary to expand its range of preclinical laboratory services, focussed on improving the prediction of drug safety, efficacy and DMPK. David has numerous publications in respiratory and vascular pharmacology, including a number of reviews on the state of the art in human tissue research and its application to drug development and personalised medicine. He is on the Board of the newly formed Stratified Medicines Scotland Innovation Centre and is an active contributor to the drive towards greater use of human tissues in drug development..

Presentation abstracts

A vision for funded collections of human tissue and biosamples

Jon Fistein, Medical Research Council

The UKCRC Experimental Medicine Funders Group has adopted a vision for funded collections of human tissue and biosamples (see http://www.ukcrc.org/research-infrastructure/experimental-medicine/funders-vision-for-human-tissue-resources/) which is to:

'…maximise the value of human tissue samples and resources while minimising duplication of effort. This requires better characterisation of tissue samples…and increased linkage to accurate clinical data. Sample collections must then be made more easily discoverable and accessible for use in high quality, ethical research'.

In order to achieve this vision the funders have highlighted the need for systems to make collections discoverable (Action 9). It also recognised the need for increased harmonisation of collection and storage of samples across academia, the NHS and industry; the importance of public engagement around tissue donation for research; and the need to make the UK more attractive for researchers.

In order to support these aims, a partnership of funders has earmarked £900k over three years to establish a Centre to develop a prototype and deliver a functional Resource Finder/Directory to enable researchers to discover, search across and contact multiple human tissue and biosample collections via a unified interface. The Centre is expected to:

- Build and manage engagement between researchers, biosample collections, the public, regulators and policy makers supporting evidence-based approaches to best practice in sample collection, governance and public engagement;
- Provide coordination and guidance to increase harmonisation of standards across the entire biosample lifecycle; and
- Provide an evaluation of the system, in terms of usability, effectiveness in providing the ability to locate relevant samples, a demonstration of the benefits of the chosen approach, and an appraisal of potential options for a second phase of development, for example with increased metadata content and functionality.

The funding decision will be taken in early August 2014 with funding to begin in the autumn of 2014.

Biography

Jon is the Clinical Programme Manager at the Medical Research Council. His work at the MRC combines managing and running funding calls with providing advice on the legal and ethical issues surrounding research data use. Jon has extensive experience working in healthcare management at a senior level both in employed roles and as a consultant. He is qualified as a medical doctor and as a barrister with an LLM (Distinction). He has vocational qualifications in IT project management and has completed an Open University MBA.

Regulatory Panel Biographies

David Jones – Medicines and Healthcare Products Regulatory Agency

After spending 8 years in Contract Toxicology and 11 years as a Toxicologist in the Pharmaceutical Industry, David currently works as an Expert Pharmaco-Toxicologist within the Licensing Division of the Medicines and Healthcare products Regulatory Agency (MHRA) in London.

David's current role principally involves assessing nonclinical data for Clinical Trial Applications, both non-biological and biological. A further aspect of his job is to offer regulatory advice to companies on behalf of the MHRA or the EU's Committee for Human Medicinal Products (CHMP). David is one of the UK's accredited non-clinical experts to support the CHMP and is the UK representative on the EU's Safety Working Party (SWP). David represented the EU in the ICH revision of the M3 Guideline and on the new ICH S10 Guideline.

David is also a guest lecturer at the University of Surrey and the University of Wales and is a frequent presenter at conferences around the world.

Abby Jacobs – Food and Drug Administration

Abigail is currently an ODE Associate Director for Pharmacology/Toxicology for several Offices of Drug Evaluation (ODEs) in the Center for Drug Evaluation (CDER)/FDA. She received a B.S. in chemistry at the U. of Michigan, Ann Arbor, and a Ph.D. in biochemistry at the U. of California, Berkeley. Her postdoctoral positions were in immunochemistry and mast cell biochemistry. She spent numerous years working for the National Cancer Institute and NTP/ NIH, as a contractor before joining the Division of Antiviral Drug Products, CDER/FDA, as a toxicology reviewer in 1991. She was a pharmacology/toxicology supervisor for 8 years in the Division of Dermatologic drug products. She is a standing member of the CDER, FDA, Executive Carcinogenicity Assessment Committee, is chair of a number of CDER, FDA, Pharm/Tox Coordinating Committee working groups, and represents CDER/FDA on a number of working groups of the OECD, ICCVAM, and ICH. She is currently a co-chair of ICCVAM. She is the rapporteur for ICHM3(R2), which is an international harmonized guideline for pharmaceutical development, was an expert for ICHS6R1 (biologics), and FDA topic leader for ICHS10 (photosafety). She is currently topic leader for ICHS5R3, reproductive toxicity.

Markku Pasanen - External non-clinical expert (SWP, SAWP) for EMA

Markku has over 30 years' experience in studies on human foeto-placental steroid- and xenobiotic-metabolizing enzymes. The other main branch of has been to study the expression and regulation different CYP enzymes in connection of chemical-induced liver injury in different animal models and man. His present position (from 2006-) is professor of medicinal toxicology at the University of Eastern Finland, Faculty of Health Sciences, School of Pharmacy (Finland); before that for ten years as senior medical officer at the National Agency for Medicines, Helsinki (Finland). He is also an external non-clinical expert of the European Medicines Agency (EMA) being nominated as a member in CHMP Scientific Advice Working Party (SAWP), Safety (SWP), and Pharmacogenetics (PgWP).

Poster Abstracts

Tissue engineered 3D co-cultures as models of the nervous system

Caitriona O'Rouke

University College London

Our research uses tissue engineering technology to organise nervous system cells within hydrogels to create physiologically relevant engineered neural tissue equivalents. The models resemble functional nervous system tissue, with aligned tracts of neurons and glia arranged within a robust collagen hydrogel.

This approach, unlike more traditional cultures or those that use stiff 3D matrices or more chemically complex hydrogels, enable neural cells to behave like their *in vivo* counterparts. For example, glial cells can adopt a less reactive phenotype reminiscent of the undamaged CNS. When stimulated with a damage cue, they undergo reactive gliosis and mimic the cellular and molecular responses characteristic of CNS injury. Neuronal survival, phenotype, neurite outgrowth, degeneration and electrophysiology can be studied. Furthermore, natural neuron-glial interactions such as myelination can be recreated.

The artificial neural constructs can be made using simple cell populations or more complex mixtures of neurons and glia according to requirements, and they can be aligned and stabilised, providing a robust and reproducible environment that is highly controllable and compatible with a wide range of cell and molecular analysis techniques. This simple, consistent and physiologically relevant model system, which uses a multi-well plate format can potentially be used at a scale suitable for commercial R&D. It is compatible with animal or human cells from a wide range of sources and we are keen to work with the safety assessment community to explore ways to increase adoption of these models.

For publications and more information please visit www.jamesphillps.org or contact jb.phillips@ucl.ac.uk.

Tracking the impact of iPSC technology on reduction of animal use in drug discovery and disease research

Giorgio Salvagiotto

Cellular Dynamics

Pharmaceutical companies and academic researchers rely heavily on animal use for modelling human biology. Human induced pluripotent stem cell (iPSC) technology offers access to more clinically relevant models, being able to provide virtually any cell type in the human body in unlimited quantities. This technology also enables the generation of disease- and patient-specific cells, which have been shown to recapitulate the phenotype and functional characteristics of native cells *in vitro*. A rapidly growing body of literature supports the use of human iPSC-derived cells in research and drug discovery and suggests a commitment to and shows promise for replacing routine animal use.

Here, we present case studies to illustrate the functional utility and sensitivity of iPSC-derived cells in different applications. First, we describe an *in vitro* assay to study botulinum neurotoxin (BoNT) using human iPSC-derived neurons that could serve as a replacement for the *in vivo* mouse bioassay in cosmetic and clinical applications. These neurons provide a highly sensitive system for BoNT detection and mechanistic investigations. The second study highlights the use of human iPSC-derived cardiomyocytes for cardiac safety assessment to identify liabilities earlier in the drug development process. This system offers a highly predictive and relevant *in vitro* model for measuring compound effects on cardiac electrophysiology. These cardiomyocytes are currently under examination in both pharmaceutical companies and regulatory agencies for their potential for refining animal use in toxicology and preclinical safety. Finally, we demonstrate the application of human iPSC-derived hepatocytes to uncover new insights into the pathophysiology of human cells infected with hepatitis viruses showing broad utility for infectious disease modeling and drug screening.

The data presented underscore the rapid adoption of the iPSC technology for the ability to recapitulate the expected human biology and the impact on reduction of animal use in efficacy and predictivity studies.

Using microfluidic culture conditions in a 3D co-culture model to study the effect of environmental impacts on the airway mucosa

Cornelia Blume

University of Southampton

Background: Inhalation is an increasingly popular route of drug delivery. While evaluation of the respiratory and systemic pharmacology as well as toxicology of inhaled drugs usually involves exposure of animals, these models are limited by species differences, lack of predictive capacity, throughput and costs. However, traditional *in vitro* alternatives such as epithelial cell lines that model the airway barrier are also limited, in this case by lack of differentiated features, absence of other cell types, lack of a circulation, and by their static nature.

Aim: To develop an *in vitro* 3D model of the human airway mucosa, which allows analysis of responses to environmental challenges to closely mimic the *in vivo* situation and offer the potential for pharmacological evaluations.

Methods: Using an interdisciplinary approach, we developed a microfluidic device that supplies and maintains airway epithelial cells (AECs) under flow. Human AECs were cultured at the apical side of permeable filter supports and endothelial cells on the basolateral side facing the microfluidic flow, simulating the circulation. The model was then challenged apically with Poly(I:C) to mimic a viral infection or grass pollen and the release of mediators monitored over time.

Results: In contrast to static culture, the microfluidic culture system allowed analysis of airway barrier responses in shorter time intervals and with higher sensitivity. Epithelial-derived inflammatory mediators were also shown to activate underlining endothelial cells in our 3D co-culture model. The passage of grass pollen allergens across the barrier could be detected by mass spectrometry.

Conclusion: Microfluidic culture systems provide the tools for detailed monitoring of passage of drugs across the airway mucosa and their influence on response to environmental stimuli. This dynamic 3D model offers the potential to investigate pulmonary pharmacology and toxicology under conditions that better reflect the 3D tissue micro-environment and to replace animal studies in pulmonary pharmacology.

Differentiated, multi-cellular models of the human airways

Lindsay Marshall

Aston University

Animal models may be available for many chronic and acute lung diseases, but these are time consuming and expensive to generate and often one animal model will not recapitulate all the features of the human condition. Our laboratory is therefore focussed on developing realistic *in vitro* models of human airways that will be vital in, for example, safety pharmacology testing of inhaled therapies.

We currently 'build' our models with basement membrane proteins, human pulmonary fibroblasts and human airways epithelial cells. Altering the origin of the epithelium allows us to modify the airways component such that we can model nasal, bronchial or small airways. We show that our epithelial-fibroblast co-cultures form tight, defensive barriers and secrete mucus apically. We are currently working on inclusion of other cell types into our model to increase the complexity and thus more realistically capture the multi-cellular nature of human airways. This includes using human pulmonary microvascular endothelial cells and human monocyte-derived macrophages.

We currently use these models for multiple applications- we examine the cross-talk between the multiple cell types in the human lungs following inhalation of insults, such as cigarette smoke; we use genetically manipulated cells to mimic diseases such as cystic fibrosis; we evaluate defective dead cell clearance; we look at the effects of viral and bacterial respiratory infections and we monitor cellular responses to novel drug formulations/delivery systems.

However, we are keen to exploit our model more widely. There are questions of safety and efficacy to be considered that animal models may not give a clear answer to, and where we believe that this multi-cellular approach will be successful. At this stage, we would appreciate the opportunity to work with industry and the regulators to address these unmet needs in order to make our models a valid and useful alternative to animals.

In vitro NHBE models of the human bronchial epithelium: the future of inhalation safety assessment

Zoe Prytherch

Cardiff University

One of the primary sites of exposure to airborne substances is the bronchial epithelium, which provides an initial line of defence to these foreign particles. Additional to this constant exposure to the external environment, disruption and/or re-modelling of the bronchial epithelium occurs as a result of numerous airway diseases such as, asthma and chronic obstructive pulmonary disorder (COPD). With the ever-increasing burden of lung disease, respiratory medicine must focus on better understanding the mechanisms of lung injury/repair as well as, developing more effective therapeutics. Historically, animal models have been the go-to experimental platforms in respiratory research; however, they are deficient in many key areas in their ability to mimic the human response to inhaled compounds. Therefore, a growing need exists for accurate, *in vitro* model/s of the bronchial epithelium, reflective of the human *in vivo* situation. We have developed a normal human bronchial epithelial (NHBE) model of the bronchial epithelium, cultured at an air-liquid interface (ALI) in Millipore® cell culture inserts. This NHBE model is a fully-differentiated, pseudo-stratified, muco-ciliary epithelium; containing, basal, serous, Clara, goblet and ciliated cells. The NHBE model is stable and fully developed form Days 24 – 33 in ALI culture. In an attempt to re-create a more *in vivo*-like response to inhaled xenobiotics, the NHBE model has been co-cultured with primary human hepatocytes to creating the Metabo-Lung™ a metabolising bronchial epithelial model. The Metabo-Lung™ provides a multi-organ based approach to assessing the potential toxicity of inhaled compounds.

Human cardiac slices: a novel tool for drug safety screening

Patrizia Camelliti

University of Surrey

The overwhelming majority of newly designed drugs fail because of side effects on the heart, slowing the development of therapies at great cost to the pharmaceutical industry and the public. A reason for these failures is the absence of simple but representative experimental model systems of human myocardium that can be utilised for cost effective drug screening and early identification of cardiac side effects. In recent years, human embryonic stem cell-derived or induced pluripotent stem cell-derived cardiomyocytes in monolayers/clusters have been proposed to be suitable models, but whether these cultures carry essential adult myocardial features is still debated.

Recently we have established a new experimental preparation, the human cardiac slice. Prepared from patients' biopsies necessarily removed during cardiac surgery, cardiac slices provide a simple, reproducible and relevant preparation suitable for the study of human cardiac tissue at the multicellular level. Our results show that cardiac slices retain relevant structural and functional properties of the native human heart, including tissue architecture, cell type ratio, cell distribution and coupling, extracellular matrix and electrophysiological characteristics. Importantly, human cardiac slices respond to the hERG channel blocker, E4031, with significant prolongation of action potential duration, and other arrhythmogenic events such as APD alternans, ectopic electrical activity, and conduction block, indicating that human slices can detect drug-induced arrhythmia.

We conclude that viable human cardiac slices with preserved structural, biochemical and electrophysiological properties can be prepared from adult human heart biopsies and provide a platform for safety pharmacology assessment with significant potential for reduction and partial replacement of animal research.

Comparison of mouse and human iPS cardiomyocytes models to detect cardiac safety liabilities

Aurore Colomar

UCB

Currently, drug induced cardiac safety liabilities are assessed during the early stages of drug discovery by evaluating effects on human cardiac ion channels *in vitro*, cardiovascular function *in vivo* and histopathology. We compared the ability of human (h) and mouse (m) cardiomyocytes (CM) derived from induced Pluripotent Stem cell (iPS) to detect cardiotoxicity by evaluating the effects of 7 compounds on Cell Index (CI), Beating Rate (BR) and Amplitude of Contraction (AC) of iPS-CM using an impedance based measurements (RTCA cardio, ACEA, San Diego, USA). hiPS-CM (iCell, CDI, Madison, USA) were cultured in a 96 well e-cardio Plate at 20.000 cells/well during 11 days and miPS-CM (CorAt, Axiogenesis, Köln, Germany) were cultured at 40.000 cells/well during 3 days. Both cell types were treated with 7 compounds discarded from development due to cardiac liabilities or suspected to affect cardiac activity (n≥3 wells/concentration). At the next medium change (24h for hiPS, and 16/18h for miPS) a 4h recovery period was allowed. Before treatment, hiPS-CM and miPS-CM contracted regularly at 34±6 and 116±26 beats/min (mean±SD) respectively. All compounds induced effects on h and miPS CM that were partly reversible for 6/7 compounds and irreversible at the highest concentration for 1 compound. hiPS-CM showed greater sensitivity and magnitude of response on CI, BR and amplitude compared to miPS-CM. However hiPS-CM presented more variability in control conditions than miPS-CM. In conclusion, iPS-CM seem to be an appropriate model to detect cardiotoxicity with hiPS-CM offering greater sensitivity. However, a more extensive validation using reference compounds is needed and technical issues like long duration of culture and parameter variability have to be overcome before including the human cardiomyocyte model in our pre-clinical strategy.

Use of human tissue to deliver clinically relevant assays to identify adverse effects on cardiac contractility

Mayel Gharanei

InoCardia

Adverse effect of drugs on the cardiovascular system is a major cause for compounds failing in both non-clinical and clinical studies. Some of these adverse effects may only be currently detected after the compound has been granted marketing approval and many thousands of patients treated. Such effects represent a significant issue for human health and very high costs for the pharmaceutical industry. Although adverse drug effects on the cardiovascular system may be due to many effects, one area of great concern is the effect of drugs on the force of contraction of the heart. Currently, testing for drug effects on the force of contraction of the heart is very much dependent on the use of animals (*in vivo* tests) and / or low throughput assays using isolated animal tissues (*in vitro* tests) and the predictive value of the available tests for human risk is limited. There is a need for more predictive assays with much greater throughput that can be used to select compounds with much less effect on the heart.

InoCardia is currently developing innovative commercial and technology driven *ex vivo* assays utilised to measure cardiac muscle function that is physiologically relevant, mimics *in vivo* cardiac muscle mechanics and provides a higher data content and/or throughput than current assays. Several *in vitro* isometric and force-velocity methods are currently used to investigate the effects of drug treatment on cardiac muscle contractility. However, these methods do not reflect the true biomechanics of the heart muscle.

InoCardia have shown that the human myocardial work-loop assay provides a more realistic model of heart muscle dynamics and hence that this technique may efficaciously be used to screen for the positive and negative inotropic effects of drugs, and therefore the potential cardiotoxic effects of drugs.

DrugPrint®- ion channel profiling & cardiotoxicity screening using a multicellular human cardiac model and proprietary analytical software

Scott Nicol

Midas Mediscience

The use of cultured human cardiomyocytes in conjunction with microelectrode array (MEA) technology can be employed as an *in vitro* screening tool for detecting drug-induced changes in cardiac electrophysiology (including potential for proarrhythmic interactions) but the cellular model must be robust and analysis of the extracellular action potential data can be complex and time consuming. The DrugPrint® approach combines a well-characterised multicellular human cardiac tissue model with powerful analytical pattern recognition software and provides a highly sensitive, real time analysis of the effect of drugs and chemicals, over a range of concentrations, on multiple human ion channels. The system consists of (1) a micro-electrode array (MEA), amplifier and environmental controls, that measure ion channel activity of the human cardiac tissue model, (2) a patented set of data collection, interrogation algorithms that measure drug-induced changes and (3) an interface to characterise specific effects and compare test data against a reference database. The DrugPrint® software generates a unique mathematical profile consisting of multiple features grouped into subsets that are specific for individual ion channels. Here we present reference data demonstrate that the system provides a new integrated approach for detecting drug-induced changes in cardiac electrophysiology.

Application of optical measurements of electrical activity to induced pluripotent stem cell derived cardiomyocytes as a high-throughput predictive tool for preclinical safety assessment

Tristan Pritchard-Meaker

Axiogenesis

A major concern during drug development is cardiac toxicity. The classical electrophysiological techniques, aimed to study the cardiac action potential (AP), such as the manual patch clamp and microelectrode array, are low throughput and technically demanding. Therefore the development of new technologies to improve these limitations is imperative. In this work we present the utility of CellOPTIQ (Clyde Biosciences), an optical system to detect the electrical activity of cardiac cells, in conjunction with induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs) as an alternative to overcome the bottleneck of low throughput and suitable tissue cells associated with traditional cardiac AP studies. To carry out the studies, iPSC-CMs (Axiogenesis) plated in 96 well glass bottom plate (20,000 cells/well) were transiently loaded with 6 µM di-4-ANEPPS and the cardiac electrical activity was monitored from spontaneously beating iPSC-cardiomyocytes at a data sampling rate of 10,000 Hz over 30 seconds time windows employing the platform CellOPTIQ, and the records were subsequently analysed off-line using proprietary software (Clyde Biosciences).

Directed differentiation and a transgenic approach allow for a specific selection of pure, induced pluripotent stem cell-derived cardiomyocytes. The iPSC-CMs reveal spontaneous contraction and typical physiological properties compared to their primary counterparts allowing for a multi-parametric analysis. Furthermore, iPSC-CMs can be generated in the quality, stability and amounts required in HTS.

The most relevant result was the demonstration of hERG reactivity in a murine derived iPSC-CM. A dose dependent prolongation of AP duration (APD) was caused by the well-known hERG blocker E4031, which also induced EADs and tachyarrhythmic effects at the highest concentrations employed (30 and 100 nM). The APD at 90% repolarization after incubating the cells with 10nM E4031 for 30min is 707.85±99.85 ms, representing a 237% increase above baseline (APD90 = 289.35±1.65 ms). Besides the effect on hERG channels, inhibition of L-type Ca2+ channel nifedipine was also tested. In this case a 70±10% and 50± 3%shortening of APD were shown after treating the cells with nifedipine 0.1 and 0.3 µM respectively. These results confirm the high sensitivity of optical measurements applied to iPSC-CMs to detect alterations of AP properties during the process of drug screening.

Can Cardiomyocytes-Derived From Human Induced Pluripotent Stem Cells Detect Drug-Induced Electrophysiological Effects Via Changes In Optical Action Potential Shape?

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Commercial sources of human induced pluripotent stem derived cardiac myocytes cells (HiPS-CMs) are increasingly used as a source of human cardiac cells for drug safety assessment. This study investigated the sensitivity of electrical activity of hiPS-CMs to 20 reference drugs, which are known to have cardiac effects. Optical action potentials (OAPs) were recorded using the fluorescent dye Di-4-ANEPPS in an area (0.2mm x 0.2mm) from a confluent layer of hiPS-CMs (Cellular Dynamics Inc (CDI), USA). We compared the changes in OAP shape to those found with the standard electrophysiological measurement (ECG) in the isolated rabbit left ventricular wedge (RLW). Both hiPS-CMs and RLWs detected 9 drug-induced QT-prolonging effects except for moxifloxacin where the expected effect on APs at 30μM was not observed hiPS-CMs. Both methods also detected 5 drug-induced QT-shortening effects, and RLW also detected QT-shortening related arrhythmias. In the RLW, mexiletine increased QRS-duration without having an effect on the QT-interval while it decreased the rise time of the AP in the hiPS-CMs but prolonged AP duration. Phenytoin and lidocaine increased QRS and slightly shortened QT in the RLW, but had no effects on the OAPs recorded from hiPS-CMs at equivalent concentrations.

Our data suggested that: (I) spontaneously active hiPS-CMs can be used to detect drug-induced QT-prolongation except for moxifloxacin (≤30μM) and shortening; (II) currently OAPs recorded from commercially available (CDI) spontaneously active hiPS-CMs do not reliably detect drugs that modulate Na+ channel activity.

Key words: Stem cells, Cardiomyocytes, Cardiac safety, QT-prolongation, QRS-duration, cardiac arrhythmias.

Improving the maturity of stem-cell derived cardiomyocytes: the benefits of a fully-defined culture medium

Stefan Braam, Rob Towart

Pluriomics

Many aspects of preclinical drug development have historically used animal tissue, and cells derived from human pluripotent stem cells (hPSC-CM) have already provided some possibilities for replacing certain animal tissue-based tests. In particular, hPSC-derived cardiomyocytes are providing an attractive opportunity for front-loading cardiac safety pharmacology. These cardiomyocytes express most relevant ion channels and demonstrate beating and action potentials similar to primary cardiac cells. However, when compared with primary adult cardiomyocytes, hPSC-CMs until now have remained immature, based on their membrane potentials, action potential characteristics and ill-defined ultra-structural organization.

There are now many laboratories which can produce large numbers of these cardiomyocytes, and a new initiative (Comprehensive *in vitro* Proarrhythmia Assay (CiPA)) is a novel cardiac safety screening proposal, part of which will use hPSC-derived cardiomyocytes. Intended to replace the existing regulatory strategy (ICH S7B), implementation of this initiative will replace animals used *in vivo* under the ICH S7B guidelines worldwide.

Until now, almost all laboratories producing hPSC-derived cardiomyocytes have used – have had to use – culture systems containing serum. Disadvantages are that the cells remain somewhat immature even after long periods of culture, and undefined growth hormones and/or tissue factors in the serum can give these cardiomyocytes variable characteristics.

To solve these problems, Pluriomics has developed cell culture systems free of serum and other undefined components. These novel systems support efficient differentiation and maturation of cardiomyocytes. Cardiomyocytes generated and maintained in this new culture system show enhanced sarcomeric organization, a more negative membrane resting potential and increased upstroke velocities.

We conclude that this next generation of cell culture technologies allows the generation of cardiomyocyte cells which more closely resemble human primary cells, with the additional benefit of stability in culture. In particular the avoidance of serum enables long term assays, without commonly-encountered problems such as compound plasma protein binding.

Real-time label-free cellular impedance based assessment of cardiac liability *in vitro* using human stem cell derived cardiomyocytes

Jason Gill

Durham University

Detrimental effects upon the cardiac system are a major cause of attrition during drug development, withdrawal of marketed medicines, and development of heart failure in survivors of chronic therapies. Drug-induced cardiotoxicity is a consequence of effects upon a range of molecular pathways involved with either structural or functional attributes of the heart. Current *in vitro* methodologies for assessment of drug-induced cardiotoxicity involve either sub-optimal screens involving non-cardiac cell lines engineered to express individual cardiac ion channels, or primary animal cardiomyocytes and cardiac tissue with their limited utility for *in vitro* culture and clinical translation. The recent progress in development of robust and reliable human induced pluripotent stem cell-derived cardiomyocytes and their ability to form a physiologically relevant functional syncytium with synchronous beating (contraction) *in vitro* has opened up many opportunities for improved evaluation of drug-induced cardiac liabilities. Similarly, innovative developments in microelectrode technology and electrical impedance measurement sensitivity now mean we are capable of continuous real-time detection of cell movement, such as rhythmic 'beating' of these cardiomyocytes *in vitro*. One such system, the xCELLigence Cardio real-time cell analyser (RTCA), permits label-free evaluation of cell viability simultaneously to assessment of the effect of a drug upon cardiomyocyte contractility, allowing a more robust and clinically relevant detection of functional cardiotoxic liabilities of drugs, and elucidation of their underlying molecular mechanisms. Furthermore, since impedance based monitoring can be continuously monitored and the cells are not labelled, contractility and the effects of drugs thereon can be monitored in real-time allowing pharmaco- and toxicodynamic effects to be monitored over longer timeframes, from weeks to months. This technology and its applicability will be described in this presentation.

Human proximal tubule cell monolayers provide a predictive drug safety platform

Colin Brown

Newcastle University

Animal models used in the pre-clinical, *in vivo* screening of new chemical entities (NCEs) are often poorly predictive of subsequent human toxicity, leading to substantial attrition of drug molecules successfully reaching the clinic. The kidney plays a key role in the elimination of NCEs from the body and is a target for toxicity. It is estimated that nephrotoxicity accounts for 20% of failures in preclinical development. The major roadblock to reducing drug attrition during development and identifying the potential of NCEs to cause DIN and AKI is the poorly predictive nature of current animal based screening models.

In the last 5 years we have successfully developed a Human primary proximal tubule cell monolayer model which remains differentiated on filter supports and exhibits vectorial transport of key prototypic substrates of the proximal tubule. We have used this model to decipher the renal handling of around 50 NCEs and identify important drug-drug interactions for a range of drug molecules.

We have agreed a partnership with Solvo Biotechnology to make human proximal tubule cell monolayers available to their clients and to generate a unique package of renal transport methods. Single- and double-transfected cell lines transfected with key renal transporters partnered with the Proximal Tubule cell monolayers will give a powerful holistic insight into the impact of transporters on renal clearance and/or toxicity of investigational drugs.

Determining potential human toxicity at an earlier in development, our approach allows Pharmaceutical companies to terminate drugs with an adverse profile in humans and therefore substantially reduce in *vivo* testing in rodent. There are no ethical issues associated with primary cultures from human cells, which are derived from transplant grade tissue (for which we have ethical approval for distribution to Pharmaceutical companies) and a regular supply which has the potential to produce around 80 million cells per week.

Screening in rat kidney is not an accurate predictor of digoxin handling in human kidney

Git Chung

Newcastle University

The major roadblock to reducing attrition during drug development is the poorly predictive nature of current animal-based screening models. To address this, we have developed highly predictive *in vitro* human and rat primary proximal tubule cell (PTCs) models with which we can investigate species differences in drug handling. Here we demonstrate the utility of the models by investigating the renal handling of digoxin in rat and human PTCs.

Uni-directional fluxes of digoxin (1 μM) in either the apical to basolateral (JA-B) or basolateral to apical (JB-A) direction were measured over 1 hour in either primary human or rat PTC monolayers cultured on Transwell filter supports. Fluxes were also assessed in the presence of GF120918, an MDR1 specific inhibitor, or triiodothyronine (T3), a substrate of OATPs.

In human monolayers, we observed net secretion of digoxin (JB-A 21.79 pmol/hr/cm2 >> JA-B 10.82 pmol/hr/cm2, n=3, $P<0.001$). Net secretion of digoxin was inhibited by T3 at the basolateral membrane and GF120918 at the apical membrane consistent with an OATP4C1-mediated uptake and a MDR1-mediated efflux.

In contrast, in rat monolayers, we observed a net absorption of digoxin (JB-A 21.77 pmol/hr/cm2 << JA-B 70.68 pmol/hr/cm2, $P<0.001$). Net absorption of digoxin was inhibited by T3 at the apical membrane suggesting that the high expression of Oatp transporters in the apical membrane of rat proximal tubules that are not present in human are responsible for the marked species differences in digoxin handling.

Given that a typical drug safety study design uses around a 176 animals, our models have the potential to substantially reduce animal usage by providing key data on renal handling of drugs at an early stage resulting in a better decision on whether screening in animals will be predictive of subsequent human exposure.

Tenofovir abolishes Na-dependent phosphate uptake in human proximal tubule cell monolayers

Sarah Billington

Newcastle University

Understanding the impact of drug molecules upon kidney function has been severely restricted by the lack of suitable *in vitro* models of human kidney. To address this, we have developed highly predictive *in vitro* human primary proximal tubule cell (PTCs) models with which we can investigate the interactions of drug molecules with proximal tubule cells. Here we demonstrate the power of human PTCs by identifying the mechanism by which tenofovir disrupts renal phosphate handling.

Tenofovir has been associated with hyperphosphaturia, hypophosphatemia and the onset of osteomalacia in around 30% of patients. These adverse effects have been attributed to the renal toxicity of tenofovir and decline in proximal tubule function.

Initial experiments demonstrated the expression on the apical membrane of human proximal tubule cell monolayers of the renal sodium-dependent phosphate transport (NaPi-2a). This transporter is the key transporter mediating the reabsorption of phosphate from the filtrate into the blood. The uptake of 32phosphate across the apical membrane of human PTCs was both Na+-dependent and saturable with an apparent Kd of 64.5±4.1µM. Importantly, the uptake of 32phosphate (100µM) was completely abolished by the addition of tenofovir at the apical membrane. Tenofovir inhibited phosphate uptake with an apparent Ki value of 66.3±0.9 µM.

Our data suggests that the impact of tenofovir upon renal phosphate handling does not arise from tenofovir-induced proximal tubule damage but rather from a competition between phosphate and tenofovir for NaPi-2a mediated phosphate uptake.

The abolition of phosphate uptake by tenofovir results in hyperphosphaturia and subsequently impacts plasma and bone phosphate levels. These data highlight the importance of developing holistic cell based models of the human proximal tubule. The novel mechanistic understanding of tenofovir/ phosphate interactions has been achieved *in vitro* without resort to the use of animals.

Practical considerations of using human gastrointestinal tissue for physiological experiments

John Broad

Queen Mary, University of London

Human tissue from surgical resections allows investigations of isolated tissue function whilst avoiding species-dependent differences inherent in using animal tissue. Practical considerations can limit availability of these specimens. Here we describe the process of obtaining surgical specimens to study physiology in human gastrointestinal tissue.

Following ethical agreement, identification of relevant surgeries can prove difficult. Close relationships with surgeons and theatre staff, and access to electronic surgical lists, has eased this potential hindrance. Consent, legally required to use human tissue, is obtained from patients by researchers (after training) alongside comprehensive clinical details (age, gender, disease, medications, ethnicity; Broad et al, Br J Pharmacol 2013;170:1253). Specimens are collected from several hospitals ensuring the greatest possible supply of tissues. Researchers return specimens to pathologists who confirm the tissue released for research is not required for diagnostic purposes.

Tissue is transported to the laboratory in oxygenated physiological saline solution (Krebs; room temperature), and for certain experiments can be stored (4°C) for use the following day. Following overnight storage, neuromuscular function does not significantly alter, although the time taken to achieve stable muscle contractions/relaxations to electrical field stimulation of the intrinsic nerves increases (Broad et al, Br J Pharmacol 2012;167:763). Similarly, overnight storage does not significantly alter chemical- or mechanical-induced afferent nerve firing (Maguire and Bulmer, personal communication). For the neuromuscular experiments, standardised responses are collected and matched to patient characteristics and immunohistochemical markers, providing a unique database from which retrospective analysis can be conducted (Broad et al, Gastroenterology 2014;146:5:S1:Sa2043).

This process has allowed the collection of surgical resection specimens from >400 patients since 2010. Continuous improvement has increased specimen supply (2011: 73 specimens; 2013: 127), whilst ensuring the diagnostic utility of the specimens is not compromised, allowing the use of large patient cohorts to study physiological functions of human gastrointestinal tissue.

Functional studies with human gastrointestinal tissues

Gareth Sanger

Queen Mary, University of London

In developing new medicines, studies with human tissues help understand disease and confirm the proposed medicine actually works. Thus, receptor functions in host cells or cell lines do not always reflect their functions in the native environment (e.g. motilin receptor, Sanger et al, Br J Pharmacol 2013;168:28) and although laboratory animals have much in common with humans, there are major differences in physiology (e.g. Sanger et al 2013) and pharmacology (e.g. Schattauer et al, J Biol Chem 2012;287:41595), exacerbated by strain differences (e.g. Keane et al, Nature 2011;477:289; Säfholm et al, Pulm Pharmacol Ther 2011;24:361).

Logistical and scientific obstacles hinder the study of human tissue functions. Good laboratory and clinical teamwork is required to obtain fresh tissues (Broad; this meeting). It is also necessary to minimise and understand variations among heterogeneous people and prove the translational relevance of the model by showing that drugs have relevant activities *in vitro* at appropriate concentrations.

Gastrointestinal adverse events (nausea, diarrhoea, constipation) are common among new drugs. Studies with human stomach demonstrate therapeutically-meaningful functions of different drugs which regulate gastric emptying (Broad et al, Neurogastroenterol Motil 2014;doi:10.1111/nmo.12338) or are associated with nausea (Broad et al, Gut 2012;61(suppl2):A297). Similarly, human colon can detect major differences in propensity to cause diarrhoea between cholinesterase inhibitors for Alzheimer's disease (Broad et al, Br J Pharmacol 2013;170:1253). This information is needed before examining ligands with unknown actions. It should also be routine to address patient heterogeneity by analysing data with respect to age and gender. For larger studies, data on lifestyle choices, ethnicity, past/ present disease and mediations, are important (Broad et al 2013).

Thus, functional human tissue studies explore normal human variations, an important element of safety evaluation. Such studies also champion the '3Rs' principles of animal research, in a manner which promotes the fourth 'R': Relevance.

CD3-specific antibody reduces cytokine production and alters phosphoprotein profiles in intestinal tissues from patients with inflammatory bowel disease

Anna Vossenkaemper

Queen Mary, University of London

Background & Aims: T cells mediate the development of inflammation in inflammatory bowel disease (IBD). We investigated the effects of an antibody against CD3 called otelixizumab, which induces immune tolerance, in intestinal mucosa samples from patients.

Methods: Intestinal tissues were isolated from individuals undergoing routine endoscopy or from patients undergoing intestinal surgery for colon cancer or IBD; healthy surrounding tissues were collected as controls. Isolated lamina propria mononuclear cells (LPMC) and mucosal tissue explants were incubated with otelixizumab for 24 or 48 hours. Production of inflammatory cytokines was determined by ELISA. Levels of 36 cytokines and chemokines and phosphorylation of 39 receptor tyrosine kinases and signaling molecules were measured using protein arrays. Immunoblot analysis was used to analyze T-cell transcription factors.

Results: Incubation of intestinal tissues or LPMC with otelixizumab reduced production of interferon γ , interleukin (IL)17A, and other inflammatory cytokines and chemokines, simultaneously increasing production of IL10. Mucosal biopsies from patients with IBD retained inflammation-associated tyrosine phospho-protein profiles *ex vivo*. Incubation of the inflamed tissue with otelixizumab reduced phosphorylation of these proteins to levels observed in control tissues. Otelixizumab also markedly reduced phosphorylation of proteins associated with T-cell receptor activation. Neutralization of IL10 blocked the anti-inflammatory effects of otelixizumab.

Conclusions: We observed anti-inflammatory effects of anti-CD3 in inflamed intestinal tissues from patients with IBD. The antibody appears to downregulate T-cell activation via IL10.

RIP2 kinase inhibition reduces inflammatory cytokine production in *ex vivo*-cultured inflammatory bowel disease biopsies

Anna Vossenkaemper

Queen Mary, University of London

Background and aims: The loss of epithelial barrier integrity is a common feature of inflammatory bowel disease (IBD). Disrupted barrier function allows bacteria to penetrate from the gut lumen, driving inflammation through activation of host pattern recognition receptors (PRRs). Although it is unclear which PRRs are most important in this process, accumulating evidence points to a role for the cytoplasmic PRRs NOD1 and NOD2, which signal through RIP2 kinase to activated NF-□B.

Methods: We utilized the highly potent and selective RIP2 inhibitor GSK214 to examine the function of RIP2 in spontaneous cytokine production by *ex vivo*-cultured inflamed mucosal biopsies isolated from Crohn's disease and ulcerative colitis patients. The inhibitor was added to the culture medium and explants were cultured for 24 hours. Cytokine levels were determined by ELISA.

Results: Treatment with GSK214 potently inhibited production of IL-1□, IL-6 and TNF-□ in explant cultures of inflamed mucosa. Approximately 70% of patient cultures responded to GSK214 in a dose-dependent fashion, which was similar to the response rate observed for the corticosteroid prednisilone. Data are also presented on the effect of GSK214 on activation of RIP2 as measured by Ser176 autophosphorylation.

Conclusion: Our results highlight the importance of RIP2 kinase in promoting intestinal inflammation, and suggest that RIP2 inhibitors may have therapeutic potential in the treatment of IBD.

The use of human (non- 3D equivalent) skin assays (SkimuneTM) for the detection of adverse reactions, potency and efficacy

Anne Dickinson

Alcyomics

Alcyomics has developed human *in vitro* skin explant assays (Skimune™) as alternatives to the use of animal models. They can be used as diagnostic tools for the pharmaceutical, cosmetic and chemical industries for testing of drugs, novel compounds or monoclonal antibodies for potential allergic or hypersensitivity reactions. The current favourable method to test compounds for allergenicity is the mouse local lymph node assay (LLNA). However, recent changes in EU legislation are banning animal testing on cosmetics. A number of alternative predictive test methods for identification of compounds which can cause skin sensitization are available but are inappropriate for assessment of relative potency. The Skimune™ assays have been evaluated against the LLNA, with 95% concordance ($P<0.001$ sensitivity 95%, specificity 95%) showing it is a reliable tool for safety, potency/toxicity testing and also successfully identifies chemicals which have been shown to be negative in the LLNA but positive in man e.g. nickel sulphate.

The Skimune™ technologies can also test the efficacy of novel immunomodulatory drugs or monoclonal antibodies, as well as potential allergic responses, before use in Phase I clinical trials. We have shown that the Skimune™ assays could have predicted and therefore prevented the TGN1412, Northwick Park incidence. The Skimune™ assays bridge the gap between animal-to-man studies and overcome interspecies barriers which often prevent detection of adverse effects during safety testing. The assays use a human autologous system to test for sensitivity and adverse reactions, in which activity is measured as histopathological grading of skin damage, caused by induced immune responses, which correlate with T cell proliferation and IFN-γ production. The data demonstrate that the Skimune™ technologies provide novel and reliable approaches to determination of skin sensitization, potency assessment, drug or monoclonal antibody evaluation and efficacy testing and can be used as a first step in the risk assessment process.

Long-term, human 3D co-cultured liver microtissues: characterization and applications in hepatoxicity testing

Jan Lichtenberg

InSphero

Limited translation between *in vitro* two-dimensional (2D) primary hepatic models, pre-clinical *in vivo* animal studies, and clinical trials make the accurate prediction of Drug-Induced-Liver Injury (DILI) in humans a substantial challenge. As a way to reduce animal studies, we present long-term-functional three-dimensional (3D) co-cultured human liver microtissues (MTs) and evaluate their suitability for use in drug-induced hepatotoxicity studies. 2D monolayer and 3D co-cultures of cryopreserved primary human hepatocytes were systematically assessed for viability, liver-specific morphology, and functionality. Results illustrate that the MTs have a longer (5 times) life span than the conventional 2D cultures and maintained a consistent viability and diameter. CYP3A4, CD68, CK7 and BSEP proteins were expressed in the MTs. Albumin secretion was significantly higher in the MTs. Lipopolysaccharide stimulation induced interleukin-6 secretion in the MTs over the culture period. Experimental data shows that toxic effects of long-term exposure with Troglitazone and Tolcapone were observed, but not with their non-toxic counterparts Pioglitazone and Entacapone. Taken together, the results illustrate that the MTs maintain a differentiated liver-specific phenotype for at least 4 weeks and are a valuable model to study chronic and immune-mediated DILI in humans. When these models are used to pre-screen compounds before selecting candidates for animal testing, clearly toxic compounds could be removed from the library thereby reducing the number of animal tests in the subsequent phase.

Human cell lines in genotoxicity assessment

Richard Walmsley

University of Manchester/Gentronix

The identification of genotoxic carcinogens is an important aspect of hazard identification and safety assessment. It has become clear that the use of rodent cell lines, often with '53' mutations which affect DNA repair effectiveness, produces too many positive results for non-carcinogens - false positives.

This poster describes the use of two different human cells line with functional p53 genes, and in two different contexts: the widely used GADD45a-GFP genotoxicity screening assay that identifies mutagens aneugens and clastogens, and a new mutation assay, with a readily scorable cell surface effect.

Use of human *ex vivo* skin in safety risk assessment of cosmetic ingredients.

Ruth Pendlington

Unilever Safety and Environmental Assurance Centre

Consumer safety risk assessments for cosmetic ingredients are always exposure-driven. For dermally applied ingredients, risk assessments require an understanding of the kinetics of ingredients following consumer exposure via the skin. Since the mid-1990s we have been using a model of *in vitro* (*ex vivo*) skin absorption in place of *in vivo* toxicokinetic studies in rats. This method, although preceding the OECD guidelines 428 [1] which were issued in 2004, conforms to these guidelines. The Scientific Committee for Consumer Safety (SCCS) (the Scientific Committee that provides the European Commission with opinions on health and safety risks of non-food consumer products) regard *ex vivo* human skin as the gold standard for skin absorption studies and have their own set of guidelines for the cosmetic sector [2].

The poster will include a brief description of the standard *in vitro* skin absorption method, used to generate data for the purposes of consumer safety risk assessment, as well as a description of a time course variant of the standard method; this we have developed to produce more detailed skin absorption kinetic data for the purposes of mathematical modelling, with the objective of developing an *in silico* model to predict the elicitation of skin allergy.

One of the main challenges with this work is the ability to routinely source *ex vivo* human skin to generate data for consumer safety risk assessment purposes as well as ensuring that this work complies with all appropriate safety, ethical, legal, and Corporate requirements. Latterly we have been able to source skin from commercial suppliers, working with our Local Research Ethics Committee to ensure that these suppliers adhere to the requirements of the Human Tissue Act (if tissue is sourced in the UK) or with other national regulations if sourced elsewhere. A particularly important element for ethical sourcing of human skin for this work is documentation to demonstrate that informed consent for use in non-medical research has been obtained.

[1] OECD Guideline for Testing of Chemicals, Guideline 428: Skin Absorption: *In Vitro* Method (2004).

[2] SCCS/1358/10 Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients (2010).

Current and potential use of human tissues in the agrochemical sector

Marco Corvaro, C. Terry, M. Aggarwal, J. Mehta

Dow AgroSciences

In the context of global regulatory requirements for agrochemical toxicity testing, mammalian toxicity testing has been largely based on animal testing in at least four animal species and utilisation of tissues of human origin has been limited. Current applications of human tissue are few, but these have been well established. Some examples are during new active substance testing, the evaluation of chromosome aberration in human peripheral blood lymphocytes, or the evaluation of comparative *in vitro* metabolism using human and animal tissue (particularly of liver origin) and, during formulation testing, determination of dermal absorption for the active ingredient, which may be evaluated using viable human skin (now the preferred method in certain geographies, including Europe). Despite limited use of human tissues for regulatory testing of agrochemicals in the past, these models are now being extensively utilized in investigative, mechanistic studies. These applications represent fundamental shifts, driven by Adverse Outcome Pathways (AOPs) and Mode of Action Human Relevance Frameworks (MOA/HRF), established to put toxicological findings from animal studies into context. The use of comparative gene expression, biochemical and metabolic analysis techniques using *in vitro* systems based on human liver tissue are becoming well accepted tools (i.e. use of primary hepatocytes for comparative gene expression). Potential new applications are becoming critical in safety assessment and for predictive toxicology, aiming to help address lead optimization or prioritisation of molecules for advancement. Development of these techniques, based upon a science-driven need within product safety assessment, may be pivotal in developing the techniques, tools, and scientific understanding required for reducing and refining animal testing in this sector.

Exhibitors

Exhibitors

Axion Biosystems

Axion's Multielectrode Array (MEA) systems provide a label-free *in vitro* platform for direct measurement of electrical activity in neurons and cardiomyocytes, while the efficiency and convenience of Axion's standard MEA plates facilitate use for screening-level applications. The large number of electrodes per well reveals detailed information about systems-level signal propogation.

Keywords: multielectrode arrays; stem cells; high-throughput

Axol Bioscience

Axol is a young and vibrant biotech company founded to fulfil the unmet demand for high quality human neural cells for biomedical research and discovery.

The slow productivity levels in pharmaceutical research have raised concerns that many components of the drug discovery process need to be redesigned and optimised. For example, the human immortalized cell lines or animal primary cells commonly used in traditional drug screening fail to recapitulate the pathological mechanisms of human diseases, leading to biases in assays, targets, or compounds that do not effectively address disease mechanisms. Recent advances in stem cell research, especially in the development of induced pluripotent stem cell (iPSC) technology, provide a new model for drug screening by permitting the use of human cells with the same genetic makeup as the patients without the typical quantity constraints associated with patient primary cells. Axol utlilises these technologies, providing the opportunity to derive an unlimited amount of human brain stem cells and neurons which can then generate functional brain tissue for neurological research, including in pharmaceutical efficacy and toxicology studies.

Axol products provide a convenient, timely and more cost-effective model to reduce or replace animal testing. Axol cells are versatile and can be used in a number of formats. The 96-well plated neurons for example provide a means of testing numerous conditions at a time. Importantly, these models provide a robust, consistent and reproducible system for pharmaceutical screening and are available in industrial quantities.

Keywords: human stem cells; industrial scale production; highly validated

Cellular Dynamics

Cellular Dynamics International, Inc. (CDI) is a leading developer and manufacturer of fully functioning human cells in industrial quantities to precise specifications. CDI's proprietary iCell Operating System (iCell O/S) includes true human cells in multiple cell types (iCell products), human induced pluripotent stem cells (iPSCs) and custom iPSCs and iCell products (MyCell Products). CDI's iCell O/S products provide standardized, easy-to-use, cost-effective access to the human cell, the smallest fully functioning operating unit of human biology. Customers use our iCell O/S products, among other purposes, for drug discovery and screening; to test the safety and efficacy of their small molecule and biologic drug candidates; for stem cell banking; and in the research and development of cellular therapeutics. CDI was founded in 2004 by Dr James Thomson, a pioneer in human pluripotent stem cell research at the University of Wisconsin-Madison. CDI's facilities are located in Madison, Wisconsin, with a second facility in Novato, California.

Keywords: HiPSC; disease; model

Exhibitors

InoCardia

Drug-induced adverse changes in cardiovascular function continue to be a cause of compound attrition throughout drug discovery and development. This has resulted in some pharmaceutical companies conducting an early evaluation of drug effects on the cardiovascular system to select compounds devoid of these actions. Although compound attrition due to drug effects on the heart is multifactorial it is evident that adverse drug effects on the contractility is a contributing factor. The pharmaceutical industry and regulators recognise that heart contractility assessment is currently fraught with problems with respect to human predictivity.

InoCardia has developed a range of novel clinically relevant contractility assays in order to determine the cardiovascular safety of lead compounds. InoCardia is currently developing novel human tissue and cell based assays, to assess cardiovascular liability associated with changes in cardiac contractility, that has the potential to be more predictive than existing assays.

InoCardia is the first company to show that the human trabeculae work-loop method of analysis provides a more realistic model of heart muscle dynamics and hence that this technique may effectively be used to screen for the positive and negative inotropic effects of drugs, and therefore the potential cardiotoxic effects of drugs.

Keywords: cardiovascular; safety pharmacology; contractility

InSphero

InSphero is a leading supplier of organotypic, biological *in vitro* 3D microtissues for highly predictive drug testing. The company, headquartered in Zurich, Switzerland, with subsidiaries in the USA and in Germany, currently counts all of the top ten global pharmaceutical and cosmetics companies as customers. InSphero 3D Insight™ Microtissues enable more biologically relevant *in vitro* applications in efficacy and toxicology. The portfolio covers primary liver, cardiac, brain and skin microtissues for efficacy and toxicity testing, tumor microtissues for efficacy testing in oncology and stem-cell-based models for reproductive toxicity. Aside from products for in-house use, the company also offers compound testing services. The spin-off company of the Swiss Federal Institute of Technology (ETH) Zurich and the University Zurich has been recognized for its scientific and commercial achievements with a number of national and international awards and is also certified to the ISO 9001:2008 standard for its Quality Management System.

Keywords: 3D cell culture; *in vitro* liver models; *in vitro* tumor models

Notocord

NOTOCORD® designs software for data acquisition and analysis in preclinical studies. Our company is recognized as a leading software publisher with 1500+ licenses installed worldwide in top pharmaceutical companies and research centers. You can trust our technology for your Discovery, Safety Pharmacology, Toxicology and Academic research. Our software NOTOCORD-hem™ is compatible with the main hardware manufacturers and is compliant with GLP/21 CFR Part 11 requirements. NOTOCORD®'s expertise covers cardiovascular, respiratory, electrophysiology and nervous system research areas. Our solutions are fit for preclinical R&D, including *in vivo* and *in vitro* drug testing operations, biomedical signals monitoring and research activities.

Keywords: iPSC software screening

Stemgent-Asterand

Asterand is the leading global provider of high quality, well characterized human tissue and human tissue-based research services to support drug discovery and development. We provide human-based solutions to pharmaceutical and biotechnology companies to accelerate the identification and validation of drug targets and biomarkers and to provide actionable data for the selection of drug candidates with an increased likelihood of clinical success.

Our well-established expertise has been further strengthened with Stemgent's acquisition of Asterand. Stemgent develops and markets proprietary tools and services to advance the application of human stem cells in biomedical research and drug discovery.

Keywords: human tissue research

Tissue Solutions

Tissue Solutions Ltd is an ISO 9001 accredited provider of high quality human biomaterials for pre-clinical research and offers a single access point for a wide range of diseased and normal tissues. Tissue Solutions supplies highly annotated samples from an international network ranging from direct hospital sources to niche bio banks, organ retrieval centres, charities and prospective post mortem organ and tissue collection agencies. Services include sourcing difficult to obtain samples for a range of applications such as target identification & validation, biomarker studies, companion diagnostics, pharmacogenetics and safety testing. Tissue Solutions can select on the basis of geographical and patient demographics, genetic or phenotype biomarkers, patient sub-populations and can also supply pre and post treatment samples with long term follow up data.

Keywords: human tissue procurement

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Keith Bowers	Biopta
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David Bunton	Biopta
Samuel Constant	Epithelix
David Gallacher	Janssen Research & Development
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James Louttit	GlaxoSmithKline
Hugo Vargas	Amgen
Abby Jacobs	Food and Drug Administration
David Jones	Medicines and Healthcare Products Regulatory Agency
Markku Pasanen	European Medicines Agency

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