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Postgraduate Showcase

Thursday 20th & Friday 21st September 2018
Michael Smith Lecture Theatre

Abstract Book

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Oral Presentations – Thursday 20th September

Slot	Time	Name	Topic
Opening Session & Registration			
	09:00	**Registration, Michael Smith Reception**	
	09:30	Opening Address by **Jack Lownes** Co-Chair of the Showcase Committee 2017-18	
Session One ***Chair: Kathryn McGurk***			
O1	09:45	Alex Trafford	Cancer
O2	10:00	Lekha Shah	Cancer
O3	10:15	Siyuan Tang	Medicinal Chemistry
O4	10:30	Hamza Garashi	Pharmacy Practice
O5	10:45	Emma Williams	Mental Health
O6	11:00	Karenjeet Chahal	Medicinal Chemistry
Break – 11:15-11:30			
Session Two ***Chair: Jack Lownes***			
O7	11:30	Zulfiye Yesim Turhan	Nanomaterials
O8	11:45	Estelle Yau	Pharmacokinetics
O9	12:00	Bahareh Amirloo	Biochemistry
O10	12:15	Jason Chu	Immunology
O11	12:30	Rachael Magwaza	Medicinal Chemistry
	12:45	*Flash Presentations from our Sponsors*	
Lunch and Poster Presentations – 13:00-14:30			
Session Three ***Chair: Victoria Rimmer***			
O12	14:30	Aurélie Lombard	Cancer
O13	14:45	Areti-Maria Vasilogianni	Cancer
O14	15:00	Sarah Almuhayya	Microbiology
O15	15:15	Joshua Greally	Medicinal Chemistry
O16	15:30	Wael Khawagi	Pharmacy Practice
O17	15:45	Haya Alshamri	Pharmacy Practice

Oral Presentations – Friday 21st September

Slot	Time	Name	Topic
	09:00	**Registration, Michael Smith Reception**	
Session Four ***Chair: Ali Hindi***			
O18	09:45	Sarah Alrubia	Biochemistry
O19	10:00	Eman Elkhateeb	Pharmacokinetics
O20	10:15	Carlson Carvalho Junior	Cancer
O21	10:30	Ismaeel Ramzan	Molecular Simulation
O22	10:45	Mona Alqarni	Microbiology
Break – 11:00-11:15			
Session Five ***Chair: Lisa Heaney***			
O23	11:15	Fatema Alqenae	Pharmacy Practice
O24	11:30	Thom van der Made	Biopharmaceutics
O25	11:45	James Beswick	Medicinal Chemistry
O26	12:00	Sam Chapman	Biopharmaceutics
O27	12:15	Ahmed Ashour	Pharmacy Practice
Lunch – 12:30-14:00			
Closing Session			
	14:00	Closing Address by **Victoria Rimmer** Co-Chair of the Showcase Committee 2017-18	
	14:15	Prize Presentation by **Professor Jayne Lawrence** Head of the Division of Pharmacy and Optometry	
Refreshments and Reception – 14:30-Onwards			

Poster Presentations

Poster	Name	Topic
P1	Yu Siong Ho	Analysing the factors that regulate expression of blood-brain barrier drug transporter proteins
P2	Maria Greco	Effects of excipients on protein behaviour in solution
P3	Meshal Alshakrah	Patient prioritisation for pharmaceutical care: a systematic review of assessment tools
P4	Jennifer Lang	Physiologically-based-pharmacokinetic modelling of the interplay between intestinal transporters and enzymes
P5	Ali Hindi	Examining the awareness, demand and use of community pharmacy services for people with long-term conditions
P6	Anwar Alghamdi	Understanding medication errors and adverse drug events in critically ill children
P7	Jawahir Mokhtar	Exposure to Manuka Honey Modules Antibiotic Susceptibility on Wound Isolates
P8	Victoria Rimmer	Artificial tear solution increases virulence determinants of *Pseudomonas aeruginosa* but at a fitness cost
P9	Azhar Alostad	Implementation of a herbal medicine classification system in Bahrain: Facilitators and barriers and lessons for other countries
P10	Bandar Alenezi	Changes in biomechanical properties in human eye-banked corneas following corneal cross-linking using atomic force microscopy
P11	Fai Alkathiri	Preparation and Characterisation of Graphene Oxide for Use in Biomedical Applications
P12	Livia Crica	The formation of *in vivo* protein corona on thin graphene oxide flakes
P13	Chimdi Emma-Duru	A study to determine if the iris camera can be used to monitor corneal disease and treatment outcomes
P14	Bushra Almari	Formulation of a novel 3-dimensional amyloid-β delivery system for modelling Alzheimer's disease
P15	Suzi Adams	Comparative oral taxonomic analysis using Illumina and MinION sequencing platforms
P16	Oliver Smith	Targeting the degradation of ERK5 with PROTAC technology.
P17	Norah Algazaq	Ceramides and skin inflammation: The effect of polyunsaturated fatty acid supplementation on ceramide biosynthesis in human skin cells
P18	Chen Zhao	Characterisation of metastatic potential and niche remodelling using in-vitro 3D models of breast cancer

ORAL PRESENTATIONS

O1

Examining the risk of cancer and cancer mortality in individuals with psoriasis

Alex Trafford, Parisi, RP., Kontopantelis, E., Griffiths, CEM., Ashcroft, DM.

Psoriasis is an immune-mediated skin condition affecting almost 3% of the population in the UK (1). The association between psoriasis and cancer as a comorbidity has been considered with relative frequency in the literature. However, the association remains unclear due to heterogeneity in study designs and differences in study populations covered. Furthermore, the role of psoriasis therapies and prevalent risk factors is yet to be fully elucidated. The overall aim of this PhD research programme is to investigate the association between psoriasis and cancer incidence and cancer mortality. The first objective in achieving this aim, which has been successfully completed, was to conduct a systematic review and meta-analysis of observational studies, critically evaluating and providing pooled estimates of our current understanding of the association between psoriasis and cancer. The second objective is to conduct a population-based study of cancer risk and cancer mortality in individuals with psoriasis. A systematic review and meta-analysis of observational studies concerning cancer occurrence and cancer mortality in psoriasis has been completed. Six databases were searched from inception to November 2017. Meta-analysis of included studies was conducted using random-effects models. Of 2830 returned articles, 58 were deemed eligible for inclusion. Severe psoriasis was found to be associated with all cancer occurrence and all cancer mortality. All severities of psoriasis were found to be associated with all cancer occurrence but not mortality. The occurrence of 13 site-specific cancers was found to be associated with psoriasis, whilst mortality from 3 site-specific cancers was found to be elevated in severe psoriasis. The focus of the future work of this project is to improve the understanding of the association between psoriasis and cancer, particularly with regards to lifestyle factors such as smoking. This will be achieved through a population-based cohort study using the Clinical Practice Research Datalink (CPRD) and linked hospital episode statistics, office for national statistics and cancer registry data. The use of these databases will allow for more accurate outcome and covariate ascertainment (2), bringing important improvement to the study of the association.

References:

1. Springate DA, Parisi R, Kontopantelis E, Reeves D, Griffiths CE, Ashcroft DM. Incidence, prevalence and mortality of patients with psoriasis: a U.K. population-based cohort study. The British journal of dermatology. 2017;176(3):650-8. Epub 2016/09/01.

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Metastatic potential and metastasis formation: *in vitro* 3D models to study breast cancer progression

Lekha Shah, Annalisa Tirella, Kaye Williams, Ayşe Latif

Metastasis remains a principal contributor to the poor prognosis for breast cancer [1]. Metastasis which is the development of secondary tumour growth away from the site of primary tumour is a complex phenomenon that depends on interactions of different cells types and extra cellular matrix (ECM)/niche within the tumour milieu. Particularly, the ECM is known to exert effects on cells through mechanical cues like stiffness, micro-architecture, interstitial fluid flow and chemical cues like growth factors, adhesion sites etc [2]. In fact, the role of tissue stiffness has been implied in breast cancer progression and is also considered one of the key risk factors [3]. Among different models used to delineate metastasis, 3D *in vitro* models using hydrogels (cross-linked hydrophilic polymers) as scaffold are emerging as more relevant system than 2D *in vitro* ones because they provide a controlled degree of complexity and also properties like mechanical stiffness and tissue like micro architecture. The aim of the project is hence to develop 3D *in vitro* breast cancer models that can be controlled in terms of: hydrogels composition and mechanical properties such as stiffness and permeability. With help of the developed 3D hydrogels, we want to understand the relationship of various ECM effects like matrix stiffness, composition and interstitial flow on cellular response in metastatic progression. For this, we were successful in modelling varying stiffness (0.5 kPa- 15 kPa) with Oxidized alginate and gelatin hydrogels and testing biocompatibility of the same. Further, we will characterise metastatic biomarkers expression in different breast cancer cell line (based on hormone receptor status) in response to varying matrix stiffness or composition. Metastatic potential of these cell lines in response to different matrix conditions will also be assessed by modelling extravasation and colonisation to a secondary site in a microfluidic chip/set-up. With a comprehensive data on matrix stiffness, composition and biomarker expression the developed models and findings can be further used to correlate the fingerprints with patient's prognosis and consequent therapy.

References:

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O3

Development of Stable Nicotinamide Riboside Analogues

Siyuan Tang, Sam Butterworth

In many countries, cancer is the disease that causes the second highest number of fatalities and it is predicted to become the first by surpassing cardiovascular diseases in the near future.[1] Energy metabolism reprogramming is defined as an emerging cancer hallmark and due to the Warburg Effect, cancer cells prioritise to produce their energy resources, ATP, through glycolysis even under normoxic conditions.[2,3] As a result, glycolysis is a potential promising target in cancer therapy.

Benzamide riboside (BR) was designed as an NAD^+/NADH competitor and aimed to target enzymes applying NAD^+/NADH as co-enzyme, such as LDH and PHGDH. However, several modifications are still needed on BR to further enhance its enzymes binding affinity and selectivity. For example, to mimic the NAD^+/NADH structure in LDH, hydrogen bond donor in the *para*-position of the aryl ring is applied, therefore block the amide conformation as required by target enzymes through forming the intramolecular hydrogen bond.

We have achieved the aim of synthesising most of the designed amide conformation block compounds and two more new structure have been proposed, pyrimidine and di-fluorine BR analogues, aimed at enhancing their enzymes binding affinity through space hindrance. 'ProTide' technology maybe applied on all the synthesised BR analogues due to the potential recognisable issue from NRK.[4] All the BR analogues and prodrugs *via* 'ProTide' will be tested with MTT experiments in the near future.

References:

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O4

A strategy for the implementation of the 'guideline on good pharmacovigilance practices (GVP) for Arab countries' in countries with nascent pharmacovigilance systems: The case of Kuwait

Hamza Garashi, Ellen Schafheutle and Douglas Steinke

Over the years, adverse effects resulting from the use of medicines have attracted increased international attention with various media outlets frequently releasing stories highlighting this issue. In order to combat this problem and protect patients, authorities have a mandate to implement efficient policies. This is achieved through the implementation of drug safety evaluation and monitoring mechanisms throughout their entire lifecycle in the form of a pharmacovigilance system (programme). Recently there has been increased interest and awareness among Arab World countries, such as Kuwait, in developing and implementing policies enabling them to have their own national systems. In an initiative to unify pharmacovigilance practice and performance across the Arab World, the 'guideline on good pharmacovigilance practices (GVP) for Arab countries' was created with its implementation due in July 2015. Three years have passed since, yet little is known about either the extent of or the process by which it has been implemented.

The aim of this study is to develop and design the strategy/approach for the implementation of the 'guideline on GVP for Arab countries' in Kuwait, and provide recommendations for its implementation to other countries in the region with nascent pharmacovigilance systems.

A combination of theoretical frameworks relating to pharmacovigilance and policy implementation will be used to inform the study. A mixed methods approach will be employed involving different qualitative research methods including document review; in addition to interviews with key informants in three Arab countries, namely Kuwait, Oman, and Jordan. Interview participants will be represented by four population groups of different sizes and will be selected on the basis of their role within their respective countries' National Drug Authority (NDA) or National Pharmacovigilance Centre (NPVC).

The study findings will be framed around the theoretical frameworks meant to inform the study to make recommendations for the formation and implementation of a pharmacovigilance policy in general and the 'guideline on GVP for Arab countries' in particular within Kuwait and countries of the Arab World with nascent systems.

O5

Using peer education to deliver a mental health promotion intervention in high schools

Emma Williams, Allison DG, Willis SC

Mental health (MH) is an important part of overall health, and therefore the promotion of mental health is essential for an individual's general well-being (1). Mental health promotion (MHP) focusses upon enhancing an individual's positive mental health (2). Young people can be vulnerable to the onset of mental health problems (3). One way to support positive mental health is through mental health promotion. In the study reported here, the aim is to develop and evaluate a peer-led, school-based MHP intervention. The intervention will be delivered by 3rd year pharmacy students, and contributes to the University of Manchester's core strategic goals of producing socially responsible graduates and of engaging with local communities.

Using the Medical Research Council guidelines for developing and evaluating complex interventions (4), this study will combine a review of the literature to establish the evidence base with stakeholder insight and pilot work completed in 2017 MHP to inform the development of a model for the intervention. Evaluation of the intervention will be in the form of MH knowledge and attitude questionnaires for both the high school pupils and the pharmacy students and self-esteem measures for the high school pupils.

To date there are no findings to report. Starting in December 2018 further feasibility testing of the process and outcome measures will be undertaken prior to further testing of the effectiveness of the intervention.

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O6

The preparation of a non-membrane associated PIP analogue to inhibit the localisation of AKT

Karenjeet Chahal, Sam Butterworth, Sally Freeman and Kaye Williams

PTEN (phosphatase and tensin homologue deleted from chromosome 10) is a tumour suppressor protein which is one of the most commonly mutated genes in many cancers.[1] The main substrate for PTEN is phosphatidylinositol-3,4,5-trisphosphate (PIP_3), which is a secondary messenger involved in various signalling functions of the cell. [2–5] PIP_3 is formed through the phosphorylation of PIP_2 by PI3K (phosphatidylinositol-3-kinase), leading to the recruitment of a cell survival kinase AKT to the cell membrane through binding to PIP_3 via its pleckstrin homology (PH) domain. Tumour cells which have lost the ability to express functional PTEN have an accumulation in the levels of PIP_3 resulting in a greater localisation of AKT at the cell membrane, increasing the activity of the kinase and subsequently tumour cell growth.[6]

We aim to prepare analogues of phosphatidylinositol with varying lipophilic chain lengths in the phosphonate group at the 1-position in order to establish their effects in cells. These compounds may be phosphorylated at the 3, 4 and 5 positions to the PIP_3-analogue inside cells to a species that inhibits the localisation of AKT at the cell membrane, decreasing kinase activity and tumour growth in PTEN-null cells. These compounds could allow for selectively targeting tumours as they could be deactivated to a PIP_2-like species in healthy cells but not in PTEN-null tumour cells.

Using previous reported protection strategies for *myo*-inositol, a protected diol has been formed.[7] Phosphorus-III/V coupling techniques have been explored for the successful phosphorylation of the 1-position of the protected inositol.

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O7

Multiresponsive Nanomaterials for Drug Delivery

Zulfiye Yesim Turhan, Farah El Mohtadi, Richard D'Arcy, Nicola Tirelli

Stimuli-responsive drug delivery systems (DDS) have been drawn attention due to enhanced control over the spatial and temporal release of encapsulated drugs[1]. Polysulfides have been widely studied as oxidant scavengers due to their remarkable properties such as their solubility switch from hydrophobic to hydrophilic upon oxidation, their ease of modulation of size and structure[2]. Amphiphilic copolymers of polysulfides form various self-assembled structures and PEG is the most commonly used hydrophilic block due to its stealth properties[3,4]. In addition, PEG and PEGylated nanostructures possess thermoresponsive behaviour[5]. In the case of light responsiveness, gold nanorods have been employed in photodynamic therapy due to ROS production upon NIR irradiation [6].

We have designed a multiresponsive DDS that is composed of a thermoresponsive poly(OEG), oxidation-responsive PPS, and light-responsive gold nanorods and hypothesized that this system will be completely soluble in aqueous environment at room temperature and will form a depot after subcutaneous injection. Upon NIR irradiation, ROS generated by gold nanorods will oxidize PPS triggering the local release of encapsulated drug in a controlled manner. When the NIR source is removed, the depot will retain its integrity and can be used for multiple doses. Once the drug release is completed, a cold patch can be applied for depot removal. For this purpose, we synthesized 4 PPS-macroRAFT agents (PMAs) to homopolymerize di(ethyleneglycol)methyl ether methacrylate via RAFT polymerization and the effect of R groups on the polymerization kinetics were evaluated. Both homo- and heteropolymers of oligoEG methacrylate monomer derivatives will be synthesized to evaluate the effect on cloud point at different concentrations and the block copolymers will be loaded on gold nanorods. The multiresponsive behavior of the block copolymers will be analysed in the presence and absence of gold nanorods.

References:

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O8

Development of a systematic strategy to integrate PK data to refine PBPK model predictions using a Bayesian approach

Estelle Yau, M. Gertz, A. Darwich, L. Aarons, A. Olivares-Morales and K. Ogungbenro

Physiologically-based pharmacokinetic (PBPK) models allow the predictions of pharmacokinetics (PK) of drugs and are widely used in drug discovery and development; linking dose to drug effects, extrapolation of dosing regimens in children or disease state, prediction of drug-drug interaction and so on. However, the multidimensional nature of PBPK models limits estimation of uncertain or unknown model parameters which leads to heterogeneous approaches for parameter estimation using PBPK models. Combining PBPK models with Bayesian methods could provide a more formal parameter estimation process[1]. However, the utility and limitations of such an approach have not been tested systematically. The aim of this project is to develop a systematic approach for incorporation of observed PK data in PBPK modelling in order to refine human PK predictions. Firstly, a feasibility analysis was conducted to identify key parameters explaining variability/uncertainty in drug distribution and to evaluate the potential for reduction of a whole body-PBPK (WBPBPK) model. Using mechanistic equations for tissue-to-plasma partition coefficient predictions (Kps)[2,3] and the WBPBPK model, a global sensitivity analysis[4] was performed for prototypical drugs (n= 1000, acids, weak bases, strong bases and neutrals). In addition, model reduction was explored on the basis of correlations between *in vivo* Kps from an actual dataset of 80 drugs in the rat[5]. It was found that lipophilicity and fraction of drug unbound (fu_p) were the most sensitive parameters for Kp predictions for different drug classes, except for strong bases where fu_p and blood:plasma ratio (B/P) were the least and most influential parameters, respectively. Similarly to drug specific parameters, the sensitivity of Kp predictions to physiological factors[6] was explored. In conclusion, this study represents the first step towards the development of a systematic Bayesian framework for PBPK parameter estimation in this project.

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O9

Design and development of novel therapeutic interventions: 'intelligent' catalytic systems for irreversible cleavage of disease relevant RNA

Bahareh Amirloo, Yaroslav Staroseletz, Marina A. Zenkova, David Clarke, Harmesh Aojula, Elena Bichenkova

RNA is engaged in all stages of cell life; particularly, in all processes involving macromolecules.[1] Antisense oligonucleotides, RNA interference, ribozymes and short interfering RNAs are main approaches to target RNA. Peptidyl-oligonucleotide conjugates (POCs) are metal-independent artificial ribonucleases, which can be synthesised using amphiphilic peptide and antisense oligonucleotide conjugated together through a covalent linkage. [2] Recent studies have indicated that, in principle, POCs are capable of multiple catalytic turnover. Nonetheless, POCs designed to date have cleaved RNA at two different extremes: (i) either sequence-specifically, but in a non-catalytic manner, or (ii) non-specifically but with high level of catalytic turnover.[3,4] Neither of these two extremes will make POCs desirable for therapeutics. Hence, it is vital to implement changes in POC structural design.

In an attempt to rectify this problem, non-complementary oligonucleotide sequences have been integrated into the POCs resulting in the formation of 2-to-5 membered bulges in the target RNA sequence. Following synthesis and purification, the conjugates have been fully characterised, and their purity has been assessed using analytical techniques. The preliminary data on the catalytic activity of these molecules against $tRNA^{Phe}$ have been obtained. The synthesised POCs demonstrated robust RNA binding. Based on the encouraging results, the presented POCs can be further developed to target microRNAs and LncRNAs for potential therapeutic applications, which will constitute the subsequent steps of this research project.

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O10

In vivo imaging of macrophage behaviour during wound healing

Jason Chu, Katherine Finegan, Kaye Williams, Kimberly Mace

Over 150 million individuals worldwide are affected by impaired wound healing. This has been particularly prevalent in the growing population of diabetics where up to 25% suffer from chronic wound pathologies such as diabetic foot ulcers, which often end in lower limb amputations. This problem continues to persist because of an incomplete understanding of the wound healing process. It has become apparent in recent decades that immune cells are crucially involved in the process and that macrophages may play an increasingly important role[1]. This project aims to specifically investigate the role of macrophages in wound healing by the innovative utilisation of PET technology to longitudinally assess its behaviour in healthy and diabetic murine wounds. This would be a significant improvement on the limitations of static snapshot methodologies to assess the role of immune cells in wound healing. This PET imaging modality will then be used to assess the *in vivo* wound response of macrophages to inflammation targeted therapies specific to the ERK5 signalling pathway. The final aim will involve assisting the development of an innovative nanobody-based PET technology to visualise M2-like populations of macrophages and their behaviour in healthy and diabetic wounds. As mentioned above, we aim to investigate the potential of targeting the ERK5 signalling pathway to improve wound healing. Previous murine studies have highlighted ERK5 as an influential pathway in mediating the infiltration and polarisation of macrophages in cancer[2]. We hypothesise that given the importance of macrophages in wounds; ERK5 signalling may bear significant therapeutic potential in the context of wound healing. This will be studied both *in vivo* in mice alongside human *in vitro* models. From our early studies we show that inhibiting ERK5 accelerates the migration of HaCaT cells, as a model of human keratinocytes, independent of its kinase activity, but does not affect proliferation. This translates from previous murine studies highlighting that loss of ERK5 improved wound healing. Moreover, our initial *in vivo* wound analysis by FACS and IHC reveal decreasing immune activity over the wound healing time-course, with a peak at day 4. This correlates with literature which describes an early inflammatory phase dominated by neutrophils and macrophages, which is shortly followed by highly active stages of resolution to reform and remodel tissue damage[3]. Future studies will focus on expanding our *in vitro* model systems, and introducing the PET technology to the *in vivo* wound model to gain a better understanding of the dynamic behaviour of macrophages in wound healing. These findings will provide novel insight and impactful research to fundamental biology of the wound healing process.

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O11

Treatment of malaria: Evaluation of furan-amidines & quinolines

Rachael Magwaza, Izzeddin Alsalahat, Soraya Alnabalsi, Buthaina Hussein, Ian Stratford, Kevin Couper, Ilaria Russo and Sally Freeman

Background: Malaria is an infectious disease caused by the parasite of the genus *Plasmodium* with human infection caused by species *falciparum, malaria, knowlesi, vivax* and *ovale* [1]. *P. falciparum* is associated with the most severe form of malaria, responsible for ~400 000 deaths/year especially in Africa. A number of antimalarial drugs are used to treat malaria however, *P. falciparum* has developed resistance to all drugs; hence, there is a great need for the development of new antimalarials.

Aims & Objectives: The furan-amidines and quinolines were originally designed to be inhibitors of NRH:quinone oxidoreductase 2 (NQO2), a potential therapeutic target in cancer chemotherapy [2]. *P. falciparum* contains a functionally similar type II NADH:dehydrogenases known as PfNDH2, therefore these compounds were tested against *Plasmodium falciparum* as novel antimalarial leads.

Results: Non-symmetrical furan-amidines and analogues (including thiophene, pyrrole, imidazole; imidate, reverse amidine), and quinolines were evaluated on *P. falciparum* 3D7 strain for asexual growth inhibition on the asynchronous culture, giving IC_{50} values of 0.197–5.6 µM and 1.42- 0.002506 ρM, respectively. The furan-amidines were also evaluated on the synchronized cultures to establish how early they inhibit the parasite growth, which showed that the compounds inhibited the ring and schizonts stages of the parasite life cycle. The furan methoxyamide prodrug was inactive on the asynchronous culture.

Summary: The high activity of the quinolines and furan-amidines against the *Plasmodium* parasites suggests that these compounds are potential leads as novel antimalarial drugs.

Future work: The mechanism of action of these compounds is still unknown; henceforth target identification will be conducted using the biotinylated probe method. *In vivo* studies of both the prodrug in parallel with the lead compound will be conducted to test the efficacy against *P. yoelii.*

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O12

Tumour size, new lesions and other markers as longitudinal endpoints in oncology studies

Aurélie Lombard, Leon Aarons, Hitesh Mistry, Kayode Ogungbenro

(Background) Drug assessment in oncology clinical trials is a real challenge, overall survival remains the gold standard for drug effect evaluation, despite its limits. Tumour size measurements can be used as a biomarker of drug efficacy to assess early response-to-treatment and patient benefit. (Aims) The aims of this project was to analyse tumour size measurements and survival data from a phase III clinical trial obtained from patients with non-small cell lung cancer treated with cisplatin alone or in combination with gemcitabine. Also to explore the effect of tumour size measurement precision and drug effect on tumour size dynamics and patient survival. (Methods) (1) Linear regression and tumour growth inhibition (TGI) models have been used to assess the precision of measurements of the same lesion performed by two operators. (2) The qualities and ability to predict drug effect on tumour size of two TGI models were compared using Claret model[1], linking drug exposure to tumour shrinkage, or using simple categorical model, in which drug effect is included as a categorical covariate. (3) A time-to-event analysis was performed to explore drug effect, demographic and disease characteristics on patient survival. The relative change in tumour size from baseline at week 10 was used (as a single time point) to predict survival. (Results) (1) For some lesions, measurements over time followed different trends, the raw measurements of the same lesion were correlated with an r^2=0.7285. The drug effect parameters estimated by the TGI model were correlated with an r^2 = 0.27. (2) A simple categorical model was able to describe tumour size dynamics and performed similarly compared to Claret model. (3) The ECOG performance status was the only variable that significantly impact patient survival. (Conclusion) (1) The “reading” factor is important to take into account as it might lead to incorrect interpretation of response-to-treatment. (2) A drug-response relationship was determined but no drug exposure-response relationship was observed. (3) The use of drug-related change in tumour size from baseline as a single time point for predicting survival was not enough to assess drug effect on survival. Future objectives will be to explore longitudinal tumour size metrics and other variables (e.g. neutrophil counts) linked to overall survival that could be used as biomarker for drug efficacy.

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O13

Disease impact on enzyme and transporter functions as well as other factors contributing to exposure variability in different types of cancer patients

Areti-Maria Vasilogianni, Jill Barber, Adam Darwich, Sheila Annie Peters, Amin Rostami-Hodjegan

Background: Cancer drugs exhibit high variability in cancer patients. Potential pharmacokinetic (PK) differences between healthy and cancer subjects were investigated using a physiologically-based pharmacokinetic (PBPK) approach [1]. Cancer patients and healthy subjects differ in many covariates that affect the PK (age, body weight, haematocrit, protein levels) [1]. The abundance of drug metabolising enzymes (DMEs) and transport proteins may also affect the PK in oncology patients, as these are down- or up-regulated in cancer [2]. For the validation of cancer effects on the PK variability, kinase inhibitors have been used, and PBPK models associated with them are encouraging for the creation of oncology populations. **Aims and objectives:** The aim is to study the impact of disease changes on enzyme and transporter abundance in the liver and gastrointestinal tract (GIT) using LC-MS/MS proteomics. The objectives are: quantification of DMEs and transporters, investigation of disease effects on PK changes driving the inter-patient variability to small molecule drugs in cancer patients, improvement of cancer population profiles in existing PBPK models. **Work accomplished to date:** literature report, research design, samples ordering, comparison of 2 software used for the analysis of LC-MS data, creation of a compound file including drugs with high inter-patient variability. **Plans for future work:** measurement of the abundance of DMEs and transporters in cancerous and normal tissues of cancer patients, and healthy tissues from healthy subjects and integration of the data in PBPK models.

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O14

Investigating bacterial interactions between members of the upper respiratory tract microbiota

Sarah Almuhayya, Gavin Humphreys, Andrew Mcbain

Background. The human microbiome is a complex and active microbial ecosystem composed primarily of bacteria, but also includes various microorganisms, such as viruses, protozoa, archaea, and fungi. Over the past ten years, understanding of the human microbiome has improved largely due to advances in metagenomics and meta-transcriptomic analysis. The upper respiratory tract (URT) microbiome has received considerably less research attention compared to the gut. Several recent studies on microbial systems of the upper respiratory tract have demonstrated negative correlations between specific members of the nasal cavity microbiota, such as is observed between *Staphylococcus aureus* and *Corynebacterium spp***.** A study performed in 2016 showed that nasal isolates of Staphylococcus lugdunensis IVK28 strain produce lugdunin, a potentially novel antibiotic that prohibits colonization by S. aureus

Aims and objectives. The aims of this of this project are to investigate the microbial ecology of the human URT in health, with emphasis on nares and nasal turbinates and to investigate and better understand the bacterial interactions within URT.

Methods. Mucosal swabs were collected separately from left and right anterior nares and left and right nasal tribunates of six healthy subjects. Nasal microorganisms were isolated using non-selective (Mueller-Hinton media, supplemented with 5% w/v horse blood) and selective (Bacitracin Chocolate media, Manitol Salt agar) media followed by Genomic DNA extraction and PCR amplification of pure sub-cultured bacteria. Subject recruitment and enrolment was approved by the University of Manchester (study reference: 2018-4100-6238).

Future work. Identification of cultured nasal bacterial isolates will be obtained by performing 16S rDNA sequencing**.** Next generation sequencing will be applied for the four nasal swabs separately to understand the deep sequencing profiles of nasal bacterial population in general. Clonality work will be performed on intra species isolates in order to determine distribution within an individual's upper respiratory tract. Screening the antagonistic agent (characterisation) in order to better understand underlying mechanism of action.

O15

Structure based design, synthesis and evaluation of Nampt inhibitors: for the development of conjugate therapies

Joshua Greally, Sam Butterworth, Alain Pluen, Annalisa Tirella

Nicotinamide phosphoribosyl transferase (Nampt) is a key protein that is prevalent in a number of biological pathways. One of the functions of Nampt has been well established as major co-factor in the synthesis of NAD via the NAD salvage pathway. Its primary role is to catalyse the conversion of Nicotinamide (NAM) and phosphoribosyl phosphate (PRPP) into Nicotinamide mono nucleotide (NMN) which is the initial and rate determining step of the NAD salvage pathway.[1]
Due to the Warburg effect, Nampt has been found to be upregulated in almost all cancers as a way of increasing production of NAD for glycolysis. This has led to the development of Nampt inhibitors as anti-cancer agents. Earlier literature has reported highly potent Nampt inhibitor (typically 1-5 nM GI50 in cancer cell lines) however; compounds have shown off- target interactions and toxicity. This high potency makes Nampt inhibitors ideal candidates for use in protein drug delivery systems.[2] Chemokines are small proteins that allow for the signalling of specific leukocytes to areas in which they are required. CCL2 (also known as monocyte chemoattractant protein 1 or MCP-1) is a chemokine able to recruit leukocytes that have surface proteins to which it can bind, in this case CCR2 and CCR4 (chemokine (C-C motif) receptor 2/4).[3] Recent studies carried out by the Butterworth group have led to the development of conjugated CCL2- payload systems by modifying the CCL2 structure to permit coupling via 'click' chemistry. This methodology has been successfully carried out in multiple examples, which constitute attaching fluorescent molecules to CCL2. Furthermore, these compounds still show internalization when introduced to CCR2 presenting THP- 1. Building on these findings, this project looks to develop CCL2- Nampt inhibitor conjugate system in which internalization of these compounds results in apoptosis of the cell. To date, this project has successfully shown the development and synthesis of derived Nampt inhibitors capable of CCL2 conjugation. Furthermore, following on from successful conjugation via 'click' chemistry these compounds have been found to induce apoptosis when introduced to THP-1 cells, giving some validation to the initial hypothesis.

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O16

Prescribing safety indicators related to mental health disorders and medications

Wael Khawagi, Richard Keers, Douglas Steinke

Background: Although prescribing errors and medication related harm may be common in patients with mental disorders, there has been limited research focusing on the development and application of prescribing indicators and inappropriate prescribing criteria to standardise the measurement of prescribing safety for this patient group.

Aim: The aim of this PhD is to develop, validate, operationalise and apply prescribing safety indicators related to mental health conditions and medications so that the burden of hazardous prescribing affecting those with mental illness may be better understood.

Methodology: The development of prescribing safety indicators initially began with a comprehensive systematic review to identify explicit mental health related prescribing quality and safety indicators from existing tools, by searching 7 electronic databases. There were no restrictions on the study design, language, setting, age group and country. All studies that developed, validated or updated a set of explicit medication-specific indicators or criteria that measured prescribing in terms of safety or quality were included. Relevant review articles and reference lists of included studies were hand searched for additional studies.

Findings: A total of 76 unique studies were included, five of which specifically focused on indicators for populations with mental illness. However, none of these studies covered all areas of hazardous prescribing. The most commonly used method to validate indicators was the Delphi method (n=36, 47.4%). A total of 1075 mental health related indicators were identified and categorised into eight types of prescribing problems. The most commonly reported was 'Potentially inappropriate prescribing considering diagnoses or conditions' (n=432, 40.2%) and the lowest was 'omission' (n=8, 0.7%). Indicators were also categorised into nine medication categories, with 'sedative, hypnotic and anxiolytics group' the most commonly reported (n=308, 28.7%).

Conclusion and future work: This is the first systematic review to identify a comprehensive list of mental health related prescribing indicators, with the findings highlighting the need for development of a new expanded suite of indicators applicable to patients with mental illness. Future work will involve refining the identified indicators with mental health pharmacists, before carrying out a Delphi consensus survey with mental health experts desgined to reach agreement and to validate a final suite of indicators. These validated indicators will then be operationalised and applied to health records to test their feasibility, and to examine the prevalence of potentially hazardous prescribing in patients with mental illness.

O17

Developing a Screening Tool to Prioritise Patients for Pharmaceutical Care in Acute Mental Health Settings

Haya Alshamri, Penny Lewis and Richard Keers

Background

Drug related problems in mental health patients are often identified and resolved by pharmacists. However, due to increasing demand and pressure in mental health settings there is a need to prioritise patient care. Tools exist in acute hospital settings that facilitate the identification of patients who are at most risk of DRPs so that pharmacy services can be directed to where they are most needed. The aim of this PhD is to develop a screening tool to prioritise patients pharmaceutical care in acute mental health settings Existing screening tools used in general hospital settings are based on multiple risk factors that are associated with an increased risk of drug related harm. To date, there has been no study of risk factors for DRPs in mental health patients. This information would be useful in the development of a prioritisation screening tool for use in mental health settings.

Methods

As a first phase of this programme of work, a systematic review is being conducted to determine risk factors associated with DRPs in mental health patients. Eight databases are used including EMBASE, MEDLINE, PsycINFO, Cochrane Library, Cumulative Index to Nursing and Allied Health Literature [CINAHL], Health Management Information Consortium [HMIC], International Pharmaceutical Abstracts [IPA], and Web of Science. Observational studies of adults with mental illness that examine the association between patient/medication related factors and DRPs using formal statistical methods and published between January 2000 and June 2018 will be included. The findings of this review will be analysed and critically evaluated to extract accurate generalizable risk factors.

Findings

15162 studies have been identified. Those studies are currently being screened for inclusion in the review.

Future work

The findings of this literature review will inform the next phase of this PhD study in which a screening tool for pharmacists working in MH settings to prioritise patients for pharmaceutical intervention will be developed.

O18

Quantification of enzymes and transporters in human intestinal tissue and methodology development of LC-MS/MS Proteomics - implications in drug metabolism and disposition

Sarah Alrubia, Amin Rostami, Jill Barber

Background: bioavailability and distribution of drugs administered orally are influenced by intestinal enzymes and transporters. These enzymes and transports differ in their concentration according to different factors. This can be explained by the difference in proteomics quantification methods by LC-MS/MS technique, the difference in abundance between disease state and healthy state,[1, 2] and the complexity of the intestinal heterogeneity.[3]

Aim: Investigating the abundance of metabolizing enzymes and transporters in different situations to enhance the quality of drug delivery and development procedure, clinical practice and the in vivo in vitro extrapolation (IVIVE) physiological based pharmacokinetic (PBPK) prediction outcomes, which will ultimately affect drugs' efficacy and safety profile.

Objectives: Study the major preparation steps (tissue fractionation, digestion, and analysis) to enable identification of sources of variability in the abundance count of proteins in the intestine tissues.

Identify the difference in enzymes and transporters expression between different intestinal segments (jejunum, ileum, and colon).

Identify the difference in the abundance of enzymes and transporters between healthy and diseased patients particularly Crohn's disease.

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O19

Utilising Physiologically Based Pharmacokinetic Modelling and Simulation for Precision Dosing in Cirrhotic Patients

Eman Elkhateeb, Amin Rostami and Adam Darwich

Cirrhosis is characterized by loss in the functional liver mass leading to a disturbance not only in hepatic metabolism but also in all other pharmacokinetic parameters; absorption, distribution as well as excretion (1). Due to the lack of dosage recommendation in this specific patient population for a large number of already approved drugs (2) and the increasing demands for inclusion of hepatic impaired patients into early phases of clinical trials prior to drug approval (3), the need for highly qualified predictive tools like Physiologically Based Pharmacokinetics (PBPK) has become apparent. This project aims to improve the predictive ability of physiological cirrhosis models for dose adjustment with the following objectives:

-Assessment of drug metabolising enzymes and transporters in cirrhotic patients.

-Investigation of the role of PBPK in dosage adjustment in patients with hepatic impairment and implications on clinical trial design.

-Modelling and simulation of a number of drugs using Simcyp® Simulator to satisfy the clinical needs in routine practice due to shortage of labelling information in cirrhotic populations.

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O20

Investigation of the effects of hypoxia and X-ray treatment on tumour cell transmigration across the blood-brain barrier

Carlson Carvalho, Kaye Williams, Jeffrey Penny

Radiotherapy remains the standard treatment for metastatic brain tumours[1]. To metastasise to the brain, malignant cells must transmigrate across the blood-brain barrier (BBB) which is composed of endothelial cells, attached to each other by tight junctions, pericytes, and astrocytes[2]. ABC transporter proteins expressed in brain endothelial cells regulate the transcellular passage of molecules[2], including many anti-cancer agents, across the BBB. Although transporter expression has been reported to be upregulated in hypoxic conditions[3], little is known of the effect of X-ray irradiation on ABC transporter expression and function. The initial aim of this study is to evaluate the effects of X-ray irradiation and hypoxia on (i) ABCB1, ABCC5 and ABCG2 drug efflux transporters functional activity in an *in vitro* model of the BBB (ii) BBB permeability and (ii) on the ability of tumour cells to penetrate the BBB. Porcine brain endothelial cells (PBECs) were successfully isolated and 8 days after seeding displayed a characteristic spindle-like morphology. Single (20 Gy) and fractionated (5 Gy x 4 days) doses of X-ray irradiation had no significant effect on PBEC viability. Single dose X-ray irradiation had no significant effect on ABCB1 and ABCG2 activity however, ABCC5 activity was significantly ($p<0.01$) lower than in control cells. Exposure of PBECs to fractionated irradiation resulted in a significant increase in, ABCB1 ($p<0.05$) and ABCC5 ($p<0.0005$) activity, whilst no significant effect on ABCG2 activity was observed. Comparison between single and fractionated regimens showed that ABCB1 ($p<0.0001$), ABCG2 ($p<0.05$) and ABCC5 ($p<0.0001$) activity was significantly increased in the fractionated regimen. Further studies will investigate the effects of X-ray irradiation and hypoxia on ABC transporter activity and expression in PBECs, BBB tightness, and transmigration of a panel of cancer cells across porcine and human *in vitro* BBB models.

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O21

Enhancing the efficiency of molecular simulation for virtual screening

Ismaeel Ramzan, Richard Bryce, Neil Burton

The drug discovery process is both time consuming and expensive. From project launch until the finished product, it may cost in excess of $1 billion and take up to 15 years to complete.[1] Computer aided drug design (CADD) can play an important role in minimizing these costs by reducing the number of potential drug molecules that need to be synthesised and tested. This includes methods such as virtual screening which use some form of computer simulation to determine how well a drug molecule binds to a target protein. In order to determine the free energy of binding one can employ molecular dynamics simulations which generate successive structures through the integration of Newtonian equations of motion as a function of time and temperature.

Molecular dynamics simulations often utilize “force fields” which take advantage of the Born-Oppenheimer approximation to treat nuclear motion independently from electronic motion. This neglect of electronic motion, hence issues such as the Heisenberg uncertainty principle, yields a fast, albeit relatively inaccurate method when compared to quantum mechanical (QM) calculations, for determining forces on atoms.

We hope to create a forcefield that runs in the same time frame as current methods however maintaining an accuracy closer to quantum calculations using machine learning. Recent work in this area has shown promise notably the work conducted by Smith et al.[2] who were able to predict energies to a high degree of accuracy after training their model on examples of structures and energies.

We will predict forces, drawing inspiration from recent advances in deep learning to design our model. More specifically we will use a neural network based on the architecture of ResNeXt[3] which at the time of writing is one of the most powerful tools in image classification. We hope that by taking advantage of this powerful architecture as well as the recent availability of large databases of QM calculations, we can more accurately simulate molecules in solution and ultimately improve the probability of correctly predicting if a potential drug molecule will bind to a target.

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O22

Does pituitary tumour apoplexy have an infective aetiology?

Mona Alqarni, Kanna Gnanalingham, Federico Roncaroli, Ruth Ledder, Gavin Humphreys

BACKGROUND

Pituitary tumour apoplexy (PTA) is a rare but life threatening clinical syndrome resulting from the sudden expansion of the sella tursica following haemorrhage or infarction of a pre-existing pituitary adenoma. Although PTA is associated with high morbidity, understanding of the underlying causes are limited with no clear concensus on the underlying pathological mechanisms or associated precipitating factors. In addition, the majority of cases of PTA occur spontaneously with no known predisposing factors. Nevertheless, PTA has been reported in up to 40% of the cases in association with certain medical conditions, interventions or medications. In a recent pilot study, PTA subjects demonstrated dysbiosis in the sinus microbiota, whereby known respiratory tract pathogens were present [1]. The absence of such bacteria from non-apoplectic controls could suggest a potential role of the microbiota in precipitating this condition.

AIM OF THE STUDY

The primary aim of the current study project is to investigate the microbiology of the sphenoid sinus and the pituitary gland in pituitary apoplexy patients in comparison to non-apoplectic, NFPA controls using next generation deep sequencing platforms. Bacterial 'biomarker' detection (or taxa significantly associated with PTA) will then be used to guide the design of specific immuno-histochemistry probes for specific bacterial antigens in pituitary and sinus biopsy specimens.

FUTUER WORK

Microbiota profiles will be generated through applying 16S rDNA amplification of sinus biopsies from PTA and control subjects using the MiSeq next generation sequencing platform. Generated 16S rDNA datasets will then be analysed using open sourced software packages (QIIME, Phyloseq) for determination of specific taxa profiles in PTA and non-apoplectic controls. Specific bacterial taxa associated with disease will be identified and used to design antigen specific immuno-histochemistry protocols for the direct images of biopsy cross sections.

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O23

Understanding Medication Errors and Medication Related Harm Immediately Following Discharge from Hospital to Community Settings

Fatema Alqenae, Douglas Steinke and Richard Keers

Background: There is a significant body of research to help understand the risks and improve medication safety associated with hospital admission from primary care. However, there is comparatively little known about the burden, nature and causes of medication errors (MEs) and medication related harm (adverse drug events or ADEs) at transition from secondary to primary care.

Aim: The aim of this PhD is to understand the burden, nature, causes and ways to improve medication safety at transition of care from secondary to primary care.

Methods: A systematic review was first conducted which aimed to identify and critically evaluate the available international evidence on the prevalence, nature, severity and risk factors of MEs and ADEs immediately following discharge from hospital to community settings. The search was carried out using the grey literature and ten databases between the dates January 1990 and December 2017. No restrictions were applied on study language, country or population. Reference lists of included studies and relevant review articles were also searched to identify citations.

Results: Fifty-nine studies met the inclusion criteria. Fifteen studies reported MEs, seventeen studies reported unintentional medication discrepancies, sixteen studies reported adverse drug reactions and twenty studies reported ADEs. The median rate of ADEs was found to be 18.9% (IQR 15.6-29) (n=18) and the median rate for MEs was found to be 46.7% (IQR 19.7-53) (n=7) for adults' and elderly patients. Heterogeneity was observed in the included studies in terms of outcome definitions and data collection methods. The most commonly reported medications associated with post hospital discharge ADEs were antibiotics, antidiabetics, analgesic medications and cardiovascular drugs.

Conclusion and future work: Medication errors and medication related harm are common immediately following transition of care from secondary to primary care. Data concerning risk factors for these events were limited and no studies were found reporting data on ADEs in the UK. Proposed future work will involve an in-depth analysis of the nature and causes of medication related incidents occurring immediately following hospital discharge as reported to the UK National Reporting and Learning System (NRLS).

O24

Can proximal tubule on-a-chip improve quantitative predictions of renal transporter drug-drug interactions? A critical analysis of currently available *in vitro* cell-based systems.

Thom van der Made, Janssen Pharmaceutica, Annett Kunze, Amin Rostami-Hodjegan and Aleksandra Galetin

The proximal tubule in the kidney expresses a range of uptake and efflux transporters and is the major site of active renal drug secretion. Transporter-mediated drug-drug interactions can significantly impact drug exposure in either plasma and/or certain organs, which may have an impact on drug safety and efficacy[(1)]. However, quantitative prediction of renal transporter-mediated drug-drug interactions in vivo remains challenging[(2)].

Currently there is a growing interest in employing novel 3D systems (e.g. kidney-on-a-chip) for toxicity screening and disease modelling. Key *in vivo* characteristics which are hypothesized to significantly contribute to the enhanced performance of novel kidney *in vitro* systems include: presence of flow, cell-cell interactions, extracellular matrix, organ linkage and recapitulation of the 3D environment. Nevertheless, these systems still need to be evaluated and validated to the current standard for mechanistic prediction of renal drug disposition and corresponding drug-drug interactions.

The presentation provides a critical overview of the impact of *in vivo* characteristics on physiological and morphological features of the proximal tubule and the impact on renal drug transporter functionality. Additionally, a critical analysis of advantages and disadvantages of 2D and 3D renal cell-based systems to evaluate transporter-mediated drug-drug interactions is outlined. Finally, modeling of *in vitro* derived data from 2D and 3D renal systems and implications on the translation of such data to *in vivo* are discussed.

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O25

Development of Small Molecule Inhibitors of the NLRP3 Inflammasome

James A. Beswick, Tessa Swanton, Halah Hammadi, Shi Yu, Jack Green, James Cook, Catherine Lawrence, David Brough, Sally Freeman

The NLRP3 (NOD-Like Receptor Pyrin domain containing protein 3) inflammasome is a multi-protein complex responsible for the processing of the proinflammatory cytokine IL-1β (interleukin-1β).[1] The NLRP3 inflammasome is implicated in a number of morbidities, including Alzheimer's disease.[2] Inhibiting the NLRP3 inflammasome could be a new approach in neuroscience drug discovery.[3] We recently showed that the fenamates, a series of NSAIDs (Non-Steroidal Anti-Inflammatory Drugs), selectively and effectively inhibit the NLRP3 inflammasome through inhibition of VRAC (Volume Regulated Anion Channel), independently of their effect on COX (cyclooxygenase) enzymes.[4]

A number of analogues (Novel VRAC compounds, NVRs) were synthesised, based on the fenamates. Alternatives to the amine linker present in the fenamates were considered, as well as a number of other alterations in order to explore the SAR (Structure Activity Relationship) of the NVR series. Favoured alterations include a bioisosteric replacement to the carboxylic acid, and an alternative to the amine linker. The leading NVRs inhibit the release of IL-1β with an IC_{50} of 0.5 - 2 μM.

Future work is planned on the NVR series, with the aim to improve potency, to assess selectivity for VRAC over COX, and to establish good pharmacokinetic properties of the lead compounds.

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O26

Evaluation of the performance of polysorbates variants on the stability of monoclonal antibody therapeutics

Sam Chapman, Alexander Golovanov, William Small and Alain Pluen

Monoclonal therapeutic antibodies (mAbs) are often prepared as liquid formulations in pre-filled syringes (PFS); however, mAbs have a tendency to aggregate which may result in an immunogenic response[1]. A common excipient of these formulations is polysorbates (PS); PS are a type of non-ionic surfactant added to inhibit the protein aggregation during shear at interfaces. In PFS, silicone oil is used to lubricate the plunger used to deliver the mAb. However, silicone oil particles evolved from pre-filled syringes increase the apparent protein aggregates count as PS is thought to contribute to the formation of silicone droplets, and, consequently have implications for quality control[2]. Antibody therapeutics are formulated with distinct pharmaceutical grades of PS which possess varying levels of peroxides and water content and different distributions of fatty acid chain length. While the use of polysorbates in antibody formulations pervades the biopharmaceutical industry, the influence of polysorbate composition on antibody stability when subjected to stress and polysorbate decomposition remains unclear. Drug development would be better informed on how to formulate antibody therapeutics by understanding the relation between polysorbates grade and mAbs stability. Initial studies involving PFS and a model protein, bovine serum albumin, were used to evaluate polysorbate performance. Characterisation of aggregation propensity following agitation using image correlation spectroscopy did not reveal any significant difference between the ability of polysorbate 20 and polysorbate 80 to limit albumin's aggregation. When varying polysorbate concentration and type, no statistical differences in silicone oil droplet distributions were observed. Measuring the diffusion coefficients of PS using NMR identified unesterified polyoxyethylene sorbitan as an impurity which does participate in the formation of micelles. Future work will involve assessing the ability for polysorbates pharmaceutical grades to outcompete model proteins at the silicone oil/water interface and at the air/water interface.

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O27

The Role of Non-Technical Skills in Community Pharmacy

Ahmed Ashour, Melinda Lyons, Denham Phipps and Darren Ashcroft

Background: In England, the number of medication errors occurring yearly has been put at 237 million (Elliott et al., 2018). In the year 2016/2017, 1.01 billion items were dispensed by community pharmacies (NHS Digital, 2017), with some studies suggesting that the percentage of dispensing errors is at 3% (Franklin and O'Grady, 2007). Research into the cause of some of these errors in community pharmacy has mainly focused on retrospective analysis of incidents, rather than highlighting issues in the education and development of the pharmacists.

Aims: This PhD aims to identify and develop the non-technical skills of community pharmacists, to improve patient safety.

Method: In order to identify the Non-Technical Skills (NTS) required for community pharmacists to complete their role efficiently and safely, we propose initially conducting a task analysis on tasks pharmacists complete in practice. This will be done through producing a Hierarchical Task Analysis (HTA), using focus groups and observations with subject matter experts (i.e. community pharmacists).

Results: HTAs for different tasks that community pharmacists complete are currently being validated through observations, with tasks such as conducting the New Medicine Service (NMS) and the NHS Urgent Medicine Supply Advanced Service (NUMSAS) making up a larger set of 20 tasks that will form this framework.

Future Work: It is anticipated that the developed HTA will act as a framework to better understand the way pharmacists conduct their tasks and allow further collaborative research between HFE experts and pharmacy practice research. Next steps in this PhD will be conducting qualitative interviews with community pharmacist to identify the Non-Technical Skills that are required by them to complete their role safely and effectively, utilising the validated HTA as a foundation. Following this, gap analysis will take place and interventions will be conducted to identify the best way to ensure future community pharmacists have these skills.

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POSTER PRESENTATIONS

P1

Analysing the factors that regulate expression of blood-brain barrier drug transporter proteins

Yu Siong Ho, Jeffrey Penny

Introduction Neurological disorders pose an increasing health and economic burden to society, with an estimated 45 million cases of brain disorders at a cost to the UK of £115 billion per annum[1]. Presently, the pharmacotherapy of brain disorders is greatly impaired by the blood-brain barrier (BBB)[2]. The ATP-binding Cassette (ABC) efflux transporters, ABCB1, ABCG2 and ABCC5, expressed in the BBB, can reduce entry of some drugs into the central nervous system (CNS)[3], thereby impacting treatment efficacy. The nuclear receptors PXR, CAR and RXR are identified as master regulators of ABC efflux transporter expression in the BBB[4,5], and a better understanding of the mechanisms which regulate BBB ABC transporters may help improved delivery of therapeutic drugs to the CNS. **Methods** In this study, porcine brain capillary endothelial cells (PBECs) were isolated based on the method previously described in Rubin et al (1991). ABCB1, ABCG2 and ABCC5 transporter activities were determined by measuring intracellular accumulation of calcein, Hoechst 33342 and CMFDA (5-chloromethylfluorescein diacetate) respectively, in cells pre-treated with the ABCB1 inhibitor verapamil, ABCG2 inhibitor Ko143 or ABCC5 inhibitor MK547. The expression of PXR, CAR and RXR in PBECs was determined by Western blotting. The effects of rifampicin (PXR inducer), L-Sulforaphane (PXR inhibitor), CITCO (CAR inducer) and meclizine (CAR inhibitor) on ABCB1, ABCG2 and ABCC5 expression and activity was also investigated. **Results** In PBECs pre-treated with verapamil there was a significant increase ($P < 0.005$) in intracellular accumulation of calcein (430% of control). Similarly, there were significant ($P < 0.005$) increases in intracellular accumulation of Hoechst 33342 in cells pre-treated with Ko143 (184% of control cells) and in intracellular accumulation of CMFDA in cells pre-treated with Mk147 (348% of control cells). Western blotting confirmed expression of PXR at 50 kDa, RXR at 51 kDa and CAR at 45 kDa. Rifampicin treatment resulted in a significant increase in ABCB1 and ABCG2 transporter activity; while treatment with CITCO, meclizine, and L-sulforaphane significantly decreased the activities of ABCB1, ABCG2 and ABCC5. **Conclusion** Key drug efflux transporters (ABCB1, ABCC5 and ABCG2) and regulatory nuclear receptors (PXR, RXR and CAR) known to be expressed in the BBB *in vivo* are expressed in PBECs. Furthermore, these components of the BBB xenobiotic-sensing and detoxification system are functionally active, and future studies will employ therapeutic inhibitor and inducer drugs to gain a better understanding of the intracellular signalling pathways governing BBB drug transporter expression and activity.

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P2

Effects of excipients on protein behaviour in solution

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Lately, biologics are gaining higher importance in the pharmaceuticals portfolio for many pathologies from oncology to respiratory and autoimmune diseases. Between the 1986 and November 2014 47 mAbs were introduced on the market in the US and Europe1. The complex molecular structure of biologics is responsible for their advantages, such as high specificity and potency and disadvantages, such as physical and chemical instabilities. In particular, during formulation and development processes protein therapeutics are exposed to many stresses (acid/pH, thermal, interaction with surfaces) which often lead to physical and chemical degradations3. Therefore, proteinaceous analytical characterization is crucial during their development. Aim of this work is to establish the molecular mechanism for how excipients alter protein protein interactions (PPIs), and to understand the link to protein aggregation. Protein–protein and protein–excipients interactions are weak and transient and cannot be isolated as a single complex. As such, experimental methods for quantifying PPIs are indirect and require orthogonal approaches over a range of solutions conditions for their characterisation. One of the objective of this project was to develop a novel orthogonal method for quantify nonspecific PPIs parameters: diffusion coefficient (D) and interaction parameter (k_D) based on Taylor dispersion analysis (TDA). Method development of a new instruments that applies TDA called the Viscosizer TD for several types of samples, from simple mAbs to more complex system, such as antibody drug conjugates (ADC), was accomplished. Methods were developed for sizing, viscosity and k_D measurements. Future plans, comprise the performance of stability study of ADCs, for the investigation of both chemical and physical as a function of conjugation site and payload. Previous to the stability study, mutagenesis of the mAb, followed by the site-specific conjugation with the payload are going to be performed as well.

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P3

Patient prioritisation for pharmaceutical care: a systematic review of assessment tools

Meshal Alshakrah, Douglas Steinke, Penny Lewis

Background: Clinical pharmacy services are faced with limited resources, insufficient capacity and patients who present with increasingly complex medication regimes and morbidities. These indicate a need for the prioritisation of pharmacy services to those who most require them. Several risk assessment tools have been developed that seek to prioritise patients for pharmacy services; however, there has been no comprehensive review of the tools to date.

Aim: To provide a structured overview and description of the existing assessment tools used by hospital pharmacy that assess patient priority and/or complexity.

Methods: Systematic searches for English language publications (from 1990 to September 2017) were conducted in Embase, Medline, Scopus, International Pharmaceutical Abstracts and Web of Science. A descriptive analysis was conducted to summarise the tools.

Results: Seventeen risk assessment tools were described in 19 studies. Thirteen tools (76.5%) were developed in Europe, with the majority originating in the UK (47%). The majority of the tools (88%) were designed to identify patients at greatest risk of drug related problems and to guide appropriate pharmaceutical care. The tools include many risk factors. The most common risk factors were high-risk medication (88%), polypharmacy (76.5%) and patient age (76.5%). Ten out of 17 tools were validated.

Conclusion: None of the studies showed a measurable impact of the tool on patients, either from actual harm from the prescribed medication, or in a reduction in the number of medication and prescribing errors. The findings of this review may be useful to those wishing to introduce a tool for identifying patients in greater need of pharmacist input.

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P4

Physiologically-based-pharmacokinetic modelling of the interplay between intestinal transporters and enzymes

Jennifer Lang, Kayode Ogungbenro, Aleksandra Galetin

Background: Oral route is the most prevalent route of drug administration, however certain drugs are subject to extensive intestinal extraction by active efflux and/or metabolism which can reduce bioavailability and contribute to interactions with co-administered drug or food. Thus prediction of drug absorption is an essential step in drug development and physiologically-based-pharmacokinetic (PBPK) modelling has been widely used for predicting drug exposure.

Objectives: Firstly, we aim to develop a whole body - PBPK model to describe drug extraction from the small intestine (SI) mediated by metabolism (CYP3A4 probe midazolam) or active efflux (P-gp probe digoxin). Secondly, we aim to reduce the model complexity [2] and compare performances to full PBPK model.

Methods: The Advanced Compartmental Absorption and Transit (ACAT) model structure (n=7 compartments for the SI) [1] was implemented in Matlab® and used as the reference model (full ACAT). Three ACAT model reductions were investigated (one or two compartments for jejunum and ileum and only one compartment for duodenum). Performances were evaluated by comparing PK parameters and concentration-time profiles to the full ACAT model.

Results: In the case of midazolam, the four-compartment ACAT model (n = 2 jejunum) resulted in comparable predictions to the full ACAT (F_G = 0.56 vs 0.55) which was consistent with CYP3A4 abundance along the SI. For digoxin, predictions in the SI of the four-compartment model (n = 2 ileum) were slightly more in agreement with the full ACAT. But no significant differences were observed between the ACAT models which was probably due to its high intestinal extraction (F_A = 0.80).

Conclusion: This work highlighted that reduction of full ACAT model has to be considered on a case-by-case basis according to the drug properties and processes contributing to intestinal extraction and will be further studied for drugs undergoing metabolism and active transport.

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P5

Examining the awareness, demand and use of community pharmacy services for people with long-term conditions

Ali Hindi, Sally Jacobs, Ellen Schafheutle

The increasing population of patients with long-term conditions (LTCs) pose particular challenges for healthcare providers due to high levels of morbidity, healthcare costs and workloads in GP practices. Policy-makers have recognised the potential of community pharmacies to meet some of the needs of patients with LTCs by implementing novel community pharmacy services ("extended services"). However, there is a reasonable amount of evidence that patient awareness, demand and uptake of these extended services is low.[1]

This PhD research aims to explore reasons for this low awareness, demand and uptake of community pharmacy services. As part one, two systematic reviews of the literature were conducted. The first covered public and patient views of community pharmacies and confirmed that, apart from the traditional medicines supply function, patients and the public had low awareness of other services. The second review covered pharmacists' and GPs' views of community pharmacy services and acknowledged that pharmacists and GPs perceived a number of barriers to successful implementation and integration of pharmacy services despite the introduction of extended services; GP views were found to be underrepresented.

Part two was a qualitative focus group study which used marketing theory (7Ps marketing mix)[2] to examine the views of patients, pharmacists and GPs, to identify how community pharmacy services may be better integrated within the primary care pathway for people with LTCs. Using the 7Ps marketing mix highlighted that regularly using pharmacies for less urgent/routine services, incentivising joint working between community pharmacies and GP practices, ensuring consistency in delivery and quality of services and strategically commissioning and promoting community pharmacy services could influence utilisation and integration of community pharmacy services.

Part three will build on findings by designing a survey to assess and compare the extent the afore-mentioned factors could influence better use and integration of community pharmacy and positively impact outcomes for patients with LTCs.

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P6

Understanding medication errors and adverse drug events in critically ill children

Anwar A. Alghamdi, Richard Keers, Adam Sutherland, Darren M. Ashcroft

Introduction

Hospitalised children, and particularly paediatric intensive care unit (PICU) and neonatal intensive care unit (NICU) patients, may be at high risk from medication errors (MEs) and preventable adverse drug events (ADEs). A systematic review was conducted to identify, synthesise and critically evaluate the published peer reviewed evidence on the prevalence and nature of MEs and preventable ADEs in PICUs and NICUs in hospitals worldwide.

Method

The systematic review process involved searching seven electronic databases from 1st January 2000 through 17th July 2017 as well as the grey literature. Studies in English reporting rates of MEs or ADEs in children ≤18 years of age admitted to PICUs or NICUs were included.

Findings

Thirty-seven original studies were eligible for inclusion in this systematic review. In PICUs, MEs ranged from 5.7 to 48.8 per 100 medication orders and 6.4 to 9.1 per 1000 patient-days. In NICUs, MEs rates ranged from 5.5 to 77.9 per 100 medication orders and from 4 to 35.1 per 1000 patient-days. In both settings, prescribing and medication administration errors were found to be most commonly associated with MEs. The review identified a lack of research and consistency in published data regarding the actual medication related harm (ADEs) occurring in both settings.

Conclusion

MEs were found to be a common and persistent problem that cause harm to children admitted to PICUs and NICUs. Although achieving consensus on study methodology to support comparisons between different studies is an important recommendation arising from this research, important potential targets were identified to guide the development of remedial interventions.

Future work

A study to assess the frequency, nature, severity and preventability of ADEs in PICUs in three UK National Health Service hospitals will be carried out in 2018-19. Another study will be conducted in 2019-20 to describe and understand medication safety incidents (MEs and ADEs) in PICUs across England that were reported to the National Reporting and Learning System (NRLS).

P7

Exposure to Manuka Honey Modules Antibiotic Susceptibility on Wound Isolates

Jawahir Mokhtar, Andrew J McBain, Ruth G Ledder, Gavin Humphreys

Background. The clinical application of Manuka honey has recently gained momentum, particularly in treatment of chronic wound infections. The aim of this study is to assess the modulation of antibiotic sensitivity in a broader panel of chronic wound isolates.

Methods. Parent strains (P0) of *Staphylococcus aureus*, MRSA*, Staphylococcus. epidermidis, S. pyogenes, P. aeruginosa, Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis* were passaged ten times in the presence of sub-lethal concentrations of clinical grade Manuka honey to generate strain P10. Antibiotic sensitivity testing was performed using a combination of microdilution and disc diffusion methodologies for each parent strain of microorganisms (P0) and strains that have been modulated by manuka honey (P10 and X10).

Results. Variable changes in bacterial susceptibilities were noted following subtherapeutic exposure to honey. P10 strains of *S. epidermidis* and *S. pyogenes* exhibited a ≥4-fold decrease in their sensitivities to erythromycin and tetracycline in comparison to baseline values. Similarly, *E. coli* displayed a 4-fold reduction in susceptibilities to gentamicin following passaging with honey. In contrast, *K. pneumoniae* and *P. mirabilis* showed notable increases in susceptibility towards both ciprofloxacin and gentamicin. Most changes were shown to be transient in nature.

Conclusion. Wound isolates exposed to clinical grade Manuka honey exhibited transient changes in antibiotic profiles. The underlying mechanism and clinical implications of such changes are unclear and warrant further investigation.

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P8

Artificial tear solution increases virulence determinants of *Pseudomonas aeruginosa* but at a fitness cost

Victoria Rimmer, Curtis Dobson, Bianca Price, Philip Morgan, Carole Maldonado-Codina

The tear film is an important barrier against *Pseudomonas aeruginosa* keratitis. This study aimed to determine the effects of an artificial tear solution (ATS) on the growth and virulence of PA01 and PA9027.

ATS comprised a complex salt solution (CSS) supplemented with mucin and tear proteins including lactoferrin, lysozyme and IgG. Growth curves in TSB, CSS and ATS were performed and cultures were harvested mid-stationary phase for protease and haemolysis assays. Biofilm assays were also performed using peg lids by incubating cultures static for 24 hours.

Growth of PA01 and PA9027 was attenuated in both ATS and CSS, compared to TSB. For PA01, protease activity (cm^2/cell) was measured at $1.11x10^{-7}$ in ATS, a significant increase compared to CSS and TSB ($p<0.0001$). Haemolysis activity (cm^2/cell) was measured at $2.10x10^{-7}$ in ATS, a significant increase compared to CSS and TSB ($p<0.0001$). The % of bacteria within the biofilm reduced from 50% in TSB to 3.5% in CSS and 4% in ATS ($p<0.0001$). For PA9027, protease activity (cm^2/cell) was measured at $4.73x10^{-8}$ in ATS, a significant increase compared to CSS ($p<0.05$) and TSB ($p<0.0001$). Haemolysis activity (cm^2/cell) increased to $2.10x10^{-7}$ in ATS compared to CSS and TSB. The % of bacteria in the biofilm reduced from 45% in TSB to 3.4% in CSS and 4.3% in ATS ($p<0.0001$).

We conclude that a combination of the nutrient limited medium and tear proteins induces increased virulence determinants despite attenuated growth and biofilm formation. CSS likely leads to increased protease and haemolysis activity due to its low nutrient composition, with the presence of tear proteins increasing virulence further. These data support the assertion that exposure to ATS can prime *Pseudomonas aeruginosa* for invasion of the cornea.

P9

Implementation of a herbal medicine classification system in Bahrain: Facilitators and barriers and lessons for other countries

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Introduction Classification and regulation of herbal medicines (HM) varies considerably across the Arab world, with implications for patient safety. An important part of informing effective policy formation is to understand strengths and weaknesses of policy development and implementation in more advanced systems.

Aim As part of a wider project to inform a registration system for HMs in Kuwait, this paper aims to explore perceived barriers and facilitators of implementation of a classification policy in Bahrain, a country similar to Kuwait which does not manufacture but import all HMs.

Methods With ethics and drug regulatory authority approval, semi-structured face-to-face interviews were conducted with eight key officials involved in the implementation of the HM policy in Bahrain. Interview data were analysed using the thematic framework analysis approach.

Results Major barriers to policy implementation perceived by participants were lack of expertise in HMs, workload due to limitation in staff, lack of regular training due to insufficient financial resources and limitation in the current classification guideline due to the diversity of HM classifications worldwide. Some of major perceived facilitators were availability of a guideline which was forced by a decree, key officials personal effort and dedication, teamwork, support of higher authority and setting an adaptation period for pharmaceutical companies to comply with the new system.

Conclusion Using insights into barriers and facilitators in Bahrain, this study can inform an effective implementation process of a well-designed policy for HMs in Kuwait and other Arab countries.

P10

Changes in biomechanical properties in human eye-banked corneas following corneal cross-linking using atomic force microscopy

Bandar Alenezi, Chantal Hillarby, Susmito Biswas, Nigel Hodson, Arun Brahma, Hema Radhakrishnan

Purpose: To evaluate the biomechanical change in anterior and posterior corneal stroma induced by corneal cross-linking (CXL) treatment using atomic force microscopy (AFM).

Methods: A total of four pairs of human eye-banked corneas were used in the study. The mean age of donors was 60 years (ranging from 41 to 71 year), and included 2 females and 2 males. Corneal epithelial debridement was carried out in all 8 corneas. For each pair, the right corneas were cross-linked using accelerated CXL (9.86mW/cm2) for 9 minutes after applying riboflavin 1% solution (10 mg riboflavin-5-phosphate in 10 mL dextran 20% solution) for 30 minutes, and then riboflavin was applied at 3-minute intervals during UVA irradiation, while the left corneas served as controls and were not exposed to either UVA irradiation or riboflavin. Reduced Modulus values (MPa) at 100 & 200µm depths in the corneal stroma were measured using AFM.

Results: Mechanical testing showed that cross-linked corneas were stiffer than untreated ones, and the stiffness was greater in the anterior stroma compared to posterior stroma.

Conclusions: CXL treatment showed effectiveness in enhancing stromal strength, and the effect is concentrated in the anterior stroma with minimal impact on the posterior stroma. AFM is a useful tool to providing a better understanding of CXL treatment outcomes. It offers the ability to assess CXL effect at different locations within the corneal stroma, which will allow developing a method that is effective in estimating biomechanics after different CXL protocols.

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P11

Preparation and Characterisation of Graphene Oxide for Use in Biomedical Applications

Fai A. Alkathiri, Alexander Golovanov, John Burthem, Richard A. Bryce, Alain Pluen

The recent discovery of graphene in 2004 has attracted much attention as a promising material for drug delivery [1]. Due to its unique structure and geometry, graphene is a large polyaromatic molecule and has exceptional physicochemical properties, including high mechanical strength, a large specific surface area, and excellent biocompatibility. These properties make graphene an ideal material for some applications, including bioimaging, nanoelectronics, and platforms to transport different therapeutic agents like small drug molecules, proteins, antibodies and genes [2]. Graphene oxide (GO) is heterogeneous in its surface chemistry, and GO suspensions are also highly polydisperse in term of flake sizes; also, due to the presence of residual metal ions which may have been left from the oxidation process. The flakes characteristics were found to vary significantly between samples – even when prepared by the same group or company [3], and therefore this variation can be problematic in the biomedical applications. The present study aimed to prepare stable GO suspension and evaluate its stability using GO natural fluorescence properties. Therefore, the GO samples must be highly characterised, in terms of both flakes' size and surface chemistry, and understanding the suspension behaviour in different media, including water, saline and phosphate buffer solution. The traditional GO characterisation methods were limited, both in terms of the size and timescale. By using the fluorescence techniques, we expected to find ways that could report the lacks in the current characterisation methods. The GO samples were first characterised using the traditional techniques, before using Raster Image Correlation Spectroscopy (RICS) which is using the fluorescence of the GO flakes and provide broader size range of GO flakes. We found that none of the traditional methods used here can provide a full characterisation of GO flakes as is necessary for their use in biomedical applications. In particular, measuring the lateral dimensions of the flakes present in a preparation is obstructed due to the heterogeneity of the sample, and the size limits of the techniques used. Therefore, an alternative approach was assessed that exploited the inherent fluorescence of GO so that Raster Image Correlation Spectroscopy (RICS) could be used.

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P12

The formation of *in vivo* protein corona on thin graphene oxide flakes

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Marilena Hadjidemetriou and Kostas Kostarelos

Nanomaterials are well known to interact with biomolecules from biological fluids, leading to the formation of a biomolecular corona [1]. The protein component, protein corona (PC), has been indicated to be an important factor for the cellular uptake, biodistribution and excretion of nanomaterials [2]. Previous work in our lab on murine models has shown that PC can form around clinically used nanoparticles following their injection in the blood stream [3]. We have also widely studied the biodistribution and excretion profile of thin graphene oxide (GO) sheets after injection *in vivo* [4]. This study aimed at purifying thin GO-PC complexes from blood and urine following their intravenous administration in mice. Here, we have synthesized two types of GO, small GO (sGO) and ultra-small GO (usGO), and characterised them by various techniques. GO was then intravenously administered in CD-1 female mice and recovered from blood and urine soon after injection. To demonstrate the formation of a protein corona around thin GO sheets, GO-PC complexes were characterised by Raman spectrometry, transmission electron microscopy, protein quantification and electrophoresis. Results show the co-existance of graphene oxide nanosheets with a layer of protein origin. Our future plans include the analysis of molecular signatures in the protein corona formed on GO *via* proteomic analysis.

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P13

A study to determine if the iris camera can be used to monitor corneal disease and treatment outcomes

Chimdi Emma-Duru, Chantal Hillarby, Tariq Aslam

Background and Aims: Previous studies carried out by the Manchester group used the iris camera to study a group of patients with a diseased called Mucopolysaccharidoses (MPS); a rare metabolic disease that cause a dysfunction in the breakdown of glycosaminoglycan (GAG) by the lysosomal enzymes which causes cloudiness of the cornea due to the accumulation of these proteins in ocular tissues.

Methods: In a clinical based research, 120 participants with various forms of corneal pathology (85 males and 35 females, aged 18-79) and 65 normal controls (45 males and 20 females, aged 18-50) were recruited from the corneal clinic at the Manchester Royal Eye Hospital. Informed consent was obtained for all images taken during the study and the examination was performed under standard lighting with no form of dilation for the participant. During analysis, investigation and assessment of images involved selecting two pinpoint areas on the pupil close to the limbus margin, one superiorly and the other inferiorly and after analysis the value derived is assigned an acronym known as the corneal opacification measure (COM) which gives an estimate of the level of opacity of the cornea.

Results: Box plots showed remarkable difference COM scores between the study and control groups indicating mild, moderate and severe levels of haze. The correlation analysis showed no statistical relationship between COM score and visual acuity in keratoconus. However, in other diseased groups such as corneal dystrophy, statistical significant correlations were indicated with p-values less than 0.05 of 95% confidence interval.

Conclusion: All the groups with corneal disease gave different outcomes for COM levels when compare to normal controls as well as between each diseased groups. The iris camera has further demonstrated its capacity when it comes to estimating and measuring haze levels in various corneal diseases similar to other imaging techniques.

P14

Formulation of a novel 3-dimensional amyloid-β delivery system for modelling Alzheimer's disease

Bushra Almari, Joshua Jackson, Annalisa Tirella, Michael Harte

Introduction: The amyloid cascade hypothesis suggests that amyloid beta (Aβ) drives Alzheimer's disease (AD) pathology [(1)]. In order to better understand the mechanisms of AD, preclinical models have been developed where Aβ is infused into rodent brains either acutely with a single injection, or chronically (days) using a cannula. However, these models only mimic short-term effects. Our aim is to establish a novel model for AD research based on the injection of encapsulated Aβ-producing cells into the rat brain allowing sustained secretion of amyloid.

Methods: Chinese hamster ovary (CHO) cells expressing the human amyloid precursor protein gene (named 7PA2 cells) and wild-type cells were cultured, and conditioned media (CM) was analysed using ELISA. CM was concentrated to 100µM $A\beta_{1-42}$ and injected into the hippocampus of adult female rats. A novel object recognition (NOR) task was performed to assess cognitive deficits. Brains were collected post-mortem and synaptic markers analysed using Wes protein quantification method. Cell encapsulation was optimised and achieved by microextrusion of alginate through a nozzle altering cell concentration, alginate type, alginate concentration, nozzle size, gel flow rate, electrostatic potential, encapsulator vibration frequency and cationic gelling bath concentration.

Results: 7PA2 cells consistently secreted a mixture of amyloid species, including $A\beta_{1-42}$, over time. Rats injected with 7PA2 CM showed deficits in NOR compared with controls. Deficits in synaptic marker PSD95 were found in 7PA2 brains.
A cell concentration of 1.5million/mL and an alginate concentration of 2% (w/v) buffered with HEPES dropped in 0.5M calcium chloride provided the most stable microbeads. A nozzle size of 300µm, voltage of 1.0kV, frequency of 5500Hz run at the maximum extrusion speed provided uniform microbeads with an average diameter of 550µm and ~200 cells per microbead.

Conclusions: Amyloid species from 7PA2 cells result in a deficit in cognition. Our optimised method for the production of stable 3D microbeads containing amyloid-producing 7PA2 cells provides the first steps towards developing a chronic model for AD research.

P15

Comparative oral taxonomic analysis using Illumina and MinION sequencing platforms

Suzanne Adams, Andrew Cawley, Barry Murphy, Andrew McBain

Objective: To compare the use of Illumina short read sequencing for 16S rRNA gene amplicons and shotgun metagenomes with full length 16S rRNA gene sequencing using the Oxford Nanopore MinION long read technology on bacterial community composition.

Methods: Plaque samples were processed using primers targeting the V1-V2 region of the 16S rRNA gene and shotgun metagenomics via Illumina sequencing, and full length 16S rRNA gene sequencing using the Oxford Nanopore MinION. Illumina reads were processed using bespoke pipelines hosted on the Amazon-Cloud, utilising Kraken to classify metagenomic reads and a classification pipeline developed by the Forsyth Institute for the 16S reads. MinION reads were processed using both the EPI2ME Fastq 16S pipeline, which utilises the NCBI database, and Poretools then BLAST against HOMD. The resulting datasets were combined at the genus level and community differences assessed using beta diversity and analysis of variance (ANOVA). Differences in relative abundance were determined using a Dirichlet-Multinomial algorithm.

Results: From the beta diversity analysis, community differences were observed between the three sequencing methods ($p<0.01$). Significant differences in relative abundance were also observed between the three sequencing methods ($p<0.05$). Metagenomics based classification provided the most accurate taxonomic assessment, however the available databases are limited compared to those for 16S analysis. Illumina 16S analysis facilitated a more robust classification (99%) than MinION (average 85%) based on current observed error rates. For the MinION analysis, HOMD provided more relevant speciation for oral samples than NCBI.

Conclusions: Illumina sequencing provides accurate bacterial classification with greater depth of sequencing but is slower and less accessible. MinION sequencing provides faster, portable and easily accessible sequencing with longer reads but with higher observed error rates currently, leading to less accurate classification.

P16

Targeting the degradation of ERK5 with PROTAC technology

Oliver Smith, Sam Butterworth, Katherine Finegan

A large number of potential anti-cancer targets have been identified within the human proteome, however many of these, including transcription factors, non-scaffold proteins and non-enzymatic proteins (or non-enzymatic functions), are 'undruggable' with simple small molecules.[1] Other techniques including siRNA hold potential as therapeutics that act through elimination of disease causing proteins, although these have issues relating to delivery into the tumour cell.[2] Proteolysis-targeting chimeric (PROTAC) technology was first developed in the early 2000s, using small molecules to hijack the ubiquitin-proteasome system to enable degradation of a target protein. PROTACs are a double headed system: a ligase binding warhead bound via a linker to a targeting molecule which binds to the protein to be degraded.[1] When the PROTAC is bound it brings together the ligase in close proximately to the protein for this to become ubiquitylated and the degradation process is started. ERK5 is a kinase that regulates tumour immune signalling independently of it's enzymatic function, I will present my work on a new PROTAC that targets the degradation of ERK5 in cells, and thus replicates the knock out phenotype without the need for RNAi.

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P17

Ceramides and skin inflammation: The effect of polyunsaturated fatty acid supplementation on ceramide biosynthesis in human skin cells

Norah Algazaq, Anna Nicolaou, Alexandra Kendall, Suzanne Pilkington

Summary: Skin is a multifunctional organ and a major interface between the outer and inner- environments. It is an active site for lipid production and is, therefore, considered a great model to study lipid regulation and function. Ceramides (CER) are a highly complex group of sphingolipids that are vital for a healthy epidermal barrier and are, therefore, central in studies on cutaneous disorders and skin aging (Hannun and Obeid, 2008). Moreover, polyunsaturated fatty acids (PUFA) play a major role in regulating inflammatory responses and could modulate ceramide biosynthesis in the skin (Kendall et al., 2017). This project aims to explore CER biosynthesis in human epidermal keratinocytes and dermal fibroblasts, with a focus on the pathways mediating PUFA-CER interactions.

Aims: Our first experiment aimed to profile the main ceramide species produced by normal human epidermal keratinocytes (NHEK). The second experiment aimed to examine ceramide biosynthesis enzyme gene expression in normal human epidermal and dermal cells.

Methods: Cell pellets for human primary epidermal cells were collected and analysed using mass spectrometry to identify the main ceramide species in the epidermis. Measurement of ceramide biosynthesis enzyme gene expression in the epidermis and dermis was achieved using qPCR analysis.

Results: CER[NDS] and CER[NS] were the main species identified in control NHEK. Ceramdie enzyme gene expression analysis showed that CERS3 was more specific to the epidermis, whereas CERS4, SPTLC1 and SPTLC2 had similar expression levels in both skin layers.

Conclusion: CERS3 expression was higher in primary keratinocytes, which agrees with previous studies on ceramide synthase gene expressions in skin. Future studies will focus on investigating the effect of PUFA on ceramide biosynthesis in the epidermis and dermis.

P18

Characterisation of metastatic potential and niche remodelling using in-vitro 3D models of breast cancer

Chen Zhao, Annalisa Tirella, Kaye Williams, Ayşe Latif

Breast cancer, the most frequently diagnosed cancer in women1, has been found that the stiffness of primary tumour tissue increases during cancer progression. This is partially contributed by higher collagen content (ligand density), cross-linking density and linearized collagen in extracellular matrix2. Stiffness has been considered as one of the important factors in tumour microenvironment, because it can alter signalling pathway and further change the phenotype and the behaviour of cells. Therefore, we aim to develop a highly controlled three-dimensional in vitro system with different stiffness and ligand densities to mimic the development of primary breast tumour, which will be the first building block allowing further study of their effects to metastatic potential on different breast cancer cell lines. For this purpose, currently, we focus on the preparation of a library of materials, i.e. hydrogels, mimicking the stiffness of both healthy breast tissue and primary tumours (stiffness range: 0.2-16kPa) and controlling of ligand density. Hydrogels were formed by covalently cross-linked oxidized alginate/gelatin. Alginate was prepared with two degrees of oxidation: 35% and 50%. We hypothesised that this parameter would help in controlling the number of crosslinks formed in the hydrogels: hence attention was given to investigate the influence of oxidation degree to hydrogel stiffness. It was found that higher oxidation degree of alginate contributed to form stiffer hydrogels. To enlarge the number of variables to control stiffness and ligand density in the prepared hydrogels, gelatin concentration was also varied. For these purposes, hydrogels with final gelatin concentration of 5% wt., 7.5% wt. and 10% wt. were prepared. Results showed hydrogel stiffness increased with higher gelatin concentration. A library of hydrogels with stiffness in the range of 3 kPa to 15 kPa was obtained, which were in the desired stiffness range. In the preliminary hydrogel biocompatibility test, higher oxidation degree and high concentration of oxidized alginate showed toxicity to the cells.

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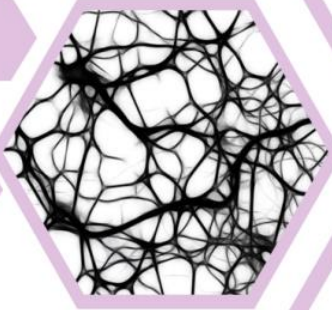
Notes

Notes



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