Annual Meeting 2023

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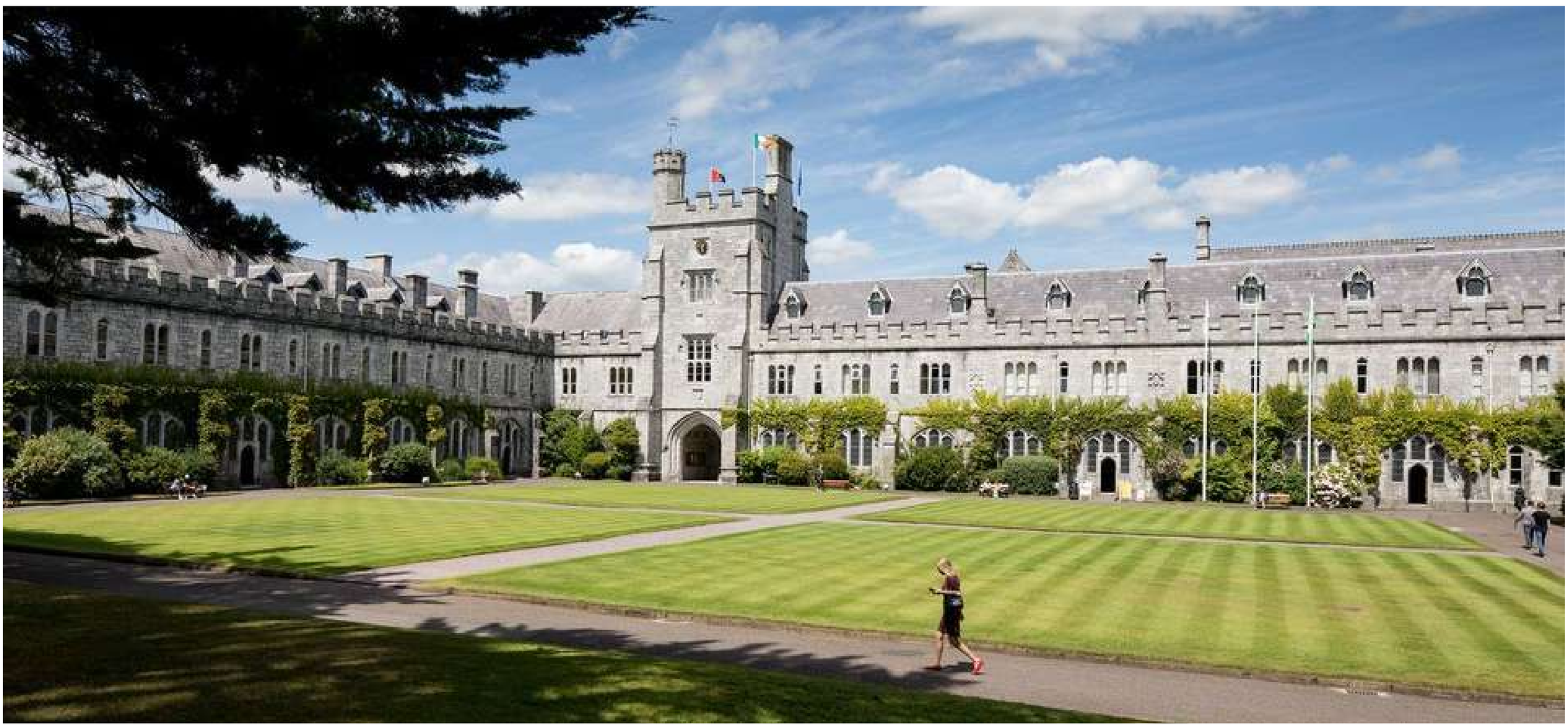
Irish Association of Pharmacologists

Book of Abstracts



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Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

BRITISH PHARMACOLOGICAL SOCIETY

Department of Pharmacology & Therapeutics

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Oral Presentations

Fawza Mashouf Alenazi, Trinity College Dublin

"VE-cadherin degradation and ubiquitination upon pharmacological inhibition of PP2A and ability to recycle by Rab11a"

Authors: Fawza Alenazi1, Ronny Schmidt2, Christoph Schroder2, Ramy Girgis2, Marco Klein2, Katrin Hufnagel2, Paul Spiers1

Background: Protein phosphatase 2A is an important enzyme in modulating the phosphorylation status of multiple proteins intrinsically linked to cellular function. Importantly, PP2A dysfunction because of pharmacological inhibition (Okadaic acid, OA) or disease disrupts blood brain barrier (BBB) function through disruption of adherent and tight junctions. Tight junction (TJ) integrity in brain endothelial cells is regulated by the integral transmembrane protein VE-cadherin. Internalization of VE-cadherin, which is associated with loosening of cell-cell junctions and disease development. The current study utilizes a PP2A inhibitor to investigate the role of VE-cadherin on blood brain barrier integrity and permeability, to delineate the mechanism by which PP2A modulates VE-cadherin in endothelial cells.

Method: The effect of OA (10nM) on the abundance and expression of VE-cadherin and PP2Ac in hCMECD3 were determined using Western blot and PCR, while PP2Ac activity was assessed using a phosphatase activity assay. Pharmacological inhibitors of the proteosome (MG132) and lysosome (chloroquine) were used to investigate mechanism underpinning VE-cadherin loss along with ubiquitination of VE-cadherin following pulldown. In collaboration with Sciomics, proteomic analysis (microarray chip) was performed following exposure to OA. On the basis of this, the role of Rab11a in VE-cadherin recycling was assessed using siRNA knockdown. The functional consequence of OA on a surrogate marker of BBB function was assessed using a permeability assay based upon FITC labelled-dextran. One-way ANOVA or the T-test/F-test were used to analyse the data ($P<0.05$ indicates significance).

Results: OA (10nM) reduced the abundance but increased mRNA expression of VE-cadherin. Interestingly, OA increased PP2Ac abundance but decreased PP2Ac activity. MG132 prevented OA-mediated loss of VE-cadherin abundance, while chloroquine had no effect. Furthermore, OA increased ubiquitination of VE-cadherin. Proteomic analysis, showed that OA differentially regulated 103 proteins, which included Rab11a and polyubiquitin. While an increase in Rab11a was not confirmed by Western blotting, its silencing exacerbated OA-mediated loss of VE-cadherin. Functionally, OA increased the permeability of an hCMECD3 monolayer to FITC-labelled dextran.

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Oral Presentations

Nicole Cosgrave , Beaumont Hospital and RCSI University of Medicine and Health Science

"Risk prediction models for adverse drug reactions and adverse drug events in older adults – A systematic review"

Authors: Nicole Cosgrave, Woei Shan Ong, Sooad Saleh, Juliane Frydenlund, David JWilliams, Caitriona Cahir

Introduction:
Adverse drug reactions (ADRs) and adverse drug events (ADEs) are common and result in significant morbidity, mortality and hospital costs. Models predicting ADR risks in older adults were previously found to lack reliability and validity [1].
Aim:
The aim of this systematic review is to expand on previous work by providing an updated, comprehensive quality assessment and analysis of ADR-risk prediction tools in older adults.
Methods:
Standard computerised databases and citations were searched (2012 to 2023) to identify relevant peer-reviewed studies. Studies which developed and/or validated an ADR/ADE prediction model for use in older adults were included. Four studies from a previous systematic review were also included [1]. The TRIPOD (transparent reporting of a multivariable prediction model for individual prognosis or diagnosis) checklist and adherence form was used to evaluate each of the included studies [2].
Results:
Six out of 11,481 titles, plus four previous studies, met all inclusion criteria and underwent TRIPOD evaluation [3-12]. Model performance was poor to good; area under the receiver operator curve (AUROC) ranged from 0.59 to 0.917. Studies had poor adherence (32-50%) to TRIPOD guidelines suggesting unfavourable investigational rigor. The studies performed poorly in terms of candidate variables, study size, missing data, external validation and implications.
Conclusion:
Eight risk prediction models were identified, exhibiting poor performance and questionable quality. This underscores the urgent need for further research in developing a validated, robust and reliable tool which, can be assessed in a real-world setting to gauge its impact and usability effectively

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Oral Presentations

Anushka Kulkarni ,The School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin

"The role of platelets in multiple myeloma"

Authors: Anushka Kulkarni , Dr. Maria Jose Santos-Martinez , Dr. Despina Bazou

Multiple myeloma (MM) is a haematological malignancy characterised by unhindered proliferation of B cells. The lack of p53 protein contributes to poor prognosis of MM patients (1). It is well established that cancer cells can aggregate platelets, a phenomenon known as Tumour-Cell-Induced-Platelet-Aggregation (TCIPA) (2). TCIPA correlates with tumour progression in solid cancers, but little is known about TCIPA in haematological malignancies. The aim of this study is to investigate the ability of MM cells with different p53 status: wild type (MM1S, KMS-27), null (JJN3) and mutant (U266), to induce TCIPA and to explore whether pharmacologically modulating these interactions can offer new insights into the clinical management of MM patients.

Methods: Platelets were isolated from healthy volunteers. TCIPA was investigated using Light-Transmission-Aggregometry (LTA). Pharmacological modulation was carried out by incubating platelets with aspirin (TXA2 inhibitor), phenanthroline (matrix metalloproteinase-MMP inhibitor), apyrase (ADP scavenger), EDTA (calcium chelating agent) and prostacyclin (cAMP modulator) prior to the addition of the different MM cell lines.

Results: KMS-27 and MM1S (wild-p53) consistently induced TCIPA, while JJN3 (null) and U226 (mutant) cells did not. Pharmacological studies revealed that EDTA and prostacyclin were able to inhibit TCIPA by KMS-27 and MM1S. However, phenanthroline modulated only KMS-27 TCIPA.

Conclusions: While p53 presence in MM cells may not be directly involved in TCIPA, phenanthroline modulation of KMS-27 TCIPA revealed that MMPs may play a significant role in this process. Further investigations exploring the role of MMPs as well as other proteins and mediators involved in TCIPA by MM cells is necessary.

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Oral Presentations

Andrea Kwakowsky , Pharmacology and Therapeutics, Galway Neuroscience Centre, School of Medicine, National University of Ireland Galway

"Investigating the therapeutic potential of targeting NKCC1 and KCC2 dysfunction in Alzheimer's Disease"

Authors: Patricia Lam, Chitra Vinnakota, Beatriz Calvo-Flores Guzmán, Julia Newland , Katie Peppercorn , Warren P. Tate , Henry J. Waldvogel , Richard L. M. Faull and Andrea Kwakowsky

Alzheimer's disease (AD) is a neurodegenerative condition. The inhibitory γ-aminobutyric acid (GABA) neurotransmitter system undergoes remodelling in AD, thus disrupting the excitatory and inhibitory (E/I) balance in the brain. The cation chloride-cotransporters, K-Cl-2 (KCC2) and N-K-Cl-1 (NKCC1), have been implicated in several neurological disorders as they affect GABA signalling polarity, but have not been explored in AD. This study examined the potential neuroprotective effects of bumetanide, an NKCC1 inhibitor, in an AD mouse model. Primary mouse hippocampal cultures were treated with beta-amyloid (Aβ1-42) and bumetanide (1μM, 10μM, 100μM, 1mM) to investigate the effect of bumetanide on cell viability. Aβ1-42 produced 53% cell death after 5 days, which did not improve with bumetanide treatment. Bumetanide at 1μM alone, and at higher concentrations, leads to 61.5 ± 1.2% cell death after 5 days, suggesting bumetanide is neurotoxic. No change in KCC2 and NKCC1 expression was observed in the in vitro AD model, however, localized NKCC1 upregulation and KCC2 downregulation were apparent in the CA1 subregion of the hippocampus in an in vivo AD mouse model. This research is questioning bumetanide's suitability for AD therapy and suggests that further investigations are required to examine whether targeting KCC2/NKCC1 might offer a therapeutic approach for AD.

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Oral Presentations

Anurag Kumar Mishra ,School of Biochemistry & Cell Biology, Biosciences Institute, University College Cork

"The Endosomal Recycling Inhibitor Primaquine Induces Ferroptosis in HER2-positive Breast Cancer Cells and Increases Lapatinib Chemosensitivity."

Authors: Anurag Mishra and Andrew J. Lindsay

A significant proportion of HER2-positive breast cancer patients develop resistance to available treatments including tyrosine kinase inhibitors (TKIs) & monoclonal antibodies, leading to a more aggressive state of the disease and poor rate of survival. It has been observed that chemo resistant cancer cells are more prone to an iron-dependent oxidative mode of cell death called ferroptosis (1). Previous work from our laboratory indicates that drug-resistant HER2-positive breast cancer cells are more vulnerable to ferroptosis and an inhibitor of the endosomal recycling pathway, primaquine (PQ), can induce ferroptosis in these cells (2). Most ferroptosis inducers suffer from poor water solubility and metabolic instability while PQ is water soluble, has good bioavailability and is FDA approved for the treatment of malaria, thus it can used as a re-purposed anti-cancer drug.

We confirmed ferroptosis induction through assessing cell rescue effects of Ferrostatin-1, increase in cellular ROS (DCFDA), lipid peroxidation (Bodipy 581/591 C11) and Golgi dispersal following treatment with PQ. Oil Red O staining & Lipid Spot 488 dye were used to determine the lipid droplet content in drug sensitive (BT474-P) and drug resistant (BT474-R) cells. The impact of lapatinib and primaquine on the cell cycle was also verified by treating BT474-P cells with the respective drugs for a period of 72 hours.

Cytotoxicity rescue experiments using a ferroptosis inhibitor (Ferrostatin-1) indicated that primaquine induces ferroptosis in both BT474-P and BT474-R cells. RPPA analysis of drug sensitive BT474 cells identified pAMPK-Thr172 and pACC-Ser79 as the primary markers for PQ-induced ferroptotic cell death. We confirmed that PQ induces ferroptosis by comparing ROS, lipid peroxidation and iron content between untreated and treated cells. We also report that PQ induced cell death and lipid peroxidation is independent of the classical ferroptosis marker GPX4. BT474-R cells have more lipid droplet content than BT474-P cells, which explains why BT474-R are more vulnerable to PQ-induced lipid peroxidation. While PQ induces a cytotoxic effect, LAP has a cytostatic effect on BT474-P cells, which was confirmed by change in the expression levels of pRbSer780 & p27.

Primaquine is a novel inducer of ferroptosis and can be readily used in combination with Lapatinib or other HER2-targeting therapies (3). In summary, in addition to our previously reported findings that PQ downregulates HER2 and HER3, we report here that PQ also induces ferroptosis in HER2-positive breast cancer cells. Further work will determine the impact of PQ on de novo lipogenesis, since fatty acid biosynthesis and uptake contribute to HER2-positive BC cell growth and resistance to anti-HER2 therapies

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Oral Presentations

Clodagh Scannell, College of Science, Engineering and Food Science, University College Cork

"Biologically based complementary and alternative medicine use is common and has the potential to cause harm in people living with and beyond cancer in Ireland "

Authors: Clodagh Scannell, Erin Stella Sullivan, Kay Curtin, Eileen O'Sullivan, Karen Matvienko-Sikar, Darren Dahly, David Robert Grimes, Derek Power, Aoife Ryan

Background: The use of biologically-based complementary and alternative medicine (BB-CAM) is prevalent among cancer survivors [1]. BB-CAM includes vitamin/mineral supplements, herbal-remedies, and special diets that have not been prescribed by a doctor/dietitian. BB-CAM use may lead to subtherapeutic/toxic drug levels of conventional cancer treatment [2]. This is caused by pharmacokinetic interactions generated by changes in the expression or functionality of CYP enzymes (2). Little is known about the use of BB-CAM in Irish oncology populations.

Methods: An online survey was designed using previous CAM literature to determine demographics, clinical characteristics and patterns of CAM before and after cancer-diagnosis. Participants were included if they were >18 years and had previously been diagnosed with cancer. Statistical analysis was conducted using SPSS(v26).

Results: There were 363 respondents, 79% female. The mean age 52 years (SD: 12 years). Breast cancer was the most common diagnosis (53%) and 67% were in remission. Having 'Ever-Used' BB-CAM in their lifetime was reported by 42%. After cancer-diagnosis, daily use of BB-CAM increased from 14% to 24% ($p<0.001$). BB-CAM users (n=97) reported using vitamin/mineral supplements (84%), dietary-supplements (78%) and herbal-remedies (50%). Perceived benefits of BB-CAM as reported by users include; to improve overall well-being (63%), reduce psychological stress (59%) and improve quality of life (55%).

Conclusion: BB-CAM use increases after cancer-diagnosis. BB-CAM has the potential to interact with conventional cancer treatment and cause harm. Healthcare professionals need to inform patients of the risks of BB-CAM and offer safer evidence-based solutions for symptom management.

Acknowledgements: This study was funded by the Irish Cancer Society research grant ASTA19RYA. Further funding was made available via Call IV of the HEA COVID Fund.

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Poster Presentations

Zahra Khan , School of Pharmacy , University College Cork

"Delivery of Tie2 mRNA to the endothelium as a novel strategy for the treatment of sepsis-induced multiple organ failure"

Authors: Lianne Mulder, Aida López Espinar, Zahra Khan, Mohamed Elkhashab, Matijs van Meurs, Jill Moser, Katie Ryan, Piotr Kowalski

Sepsis involves a dysregulated host response to infection that can lead to life-threatening multiple organ failure. The high mortality worldwide and the lack of effective treatment options urge the need to develop novel therapeutic approaches to treat sepsis. Endothelial dysfunction is considered a hallmark of sepsis to which the loss of Angpt/Tie2 signalling, a main regulator of vascular integrity, contributes significantly, making it an attractive therapeutic target[1]. The global success of the SARS-CoV19 vaccines has prompted the use of messenger RNA (mRNA) as a therapeutic modality for transient upregulation of protein expression[2] . However, intracellular delivery of mRNA remains challenging due to its high molecular weight, polyanionic nature and susceptibility to degradation by serum RNases, enforcing its formulation into delivery systems. Here, we have (1) validated the delivery of functional Tie2 mRNA and (2) developed a novel polymeric mRNA delivery system to endothelial cells [3]. Transfection of HUVECs with 5-methoxyuridine-modified Tie2 mRNA resulted in a dose-dependent increase in Tie2 protein expression up to 7-fold compared to endogenous Tie2 protein levels, and its receptor kinase activity in response to Angpt1 was confirmed. Early-onset and transient protein kinetic profile was observed favorable for therapeutic use in acute conditions. Furthermore, the formulation of a library of 36 aminopolyester-based lipid nanoparticles (APE-LNPs) with reporter FLuc mRNA identified potent APEs in vitro that exert efficient mRNA delivery beyond the liver in vivo, as mRNA translation was observed in lungs and spleen. Ultimately, we aim to explore the therapeutic potential of Tie2 mRNA to treat/prevent sepsis-induced multiple organ failure

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Poster Presentations

Maria Redmond, Pharmacology and Therapeutics, School of Medicine, University of Galway

"Characterisation of Anxiety- and Depression-Related Behaviour and the Endocannabinoid System in the Rat Hindlimb Ischemia-Reperfusion Model of Chronic Wounds"

Authors: Maria C Redmond, Catherine R Healy, Georgina Gethin, Abhay Pandit, David P. Finn

Ischemia-reperfusion injury can be an aetiology underlying the formation of chronic wounds, which are associated with a high incidence of comorbid anxiety and depression. The endocannabinoid system (ECS) may have a role in ischemia-reperfusion injury and is involved in the modulation of mood and anxiety. This study characterised anxiety- and depression-related behaviour in a rat model of hindlimb ischemia-reperfusion (HLIR) injury and investigated alterations in the ECS in discrete brain regions.

Male and female Sprague-Dawley rats (220-350g, n=11-12/group) underwent HLIR injury or sham procedure on the left hind limb. Anxiety-related behaviour was assessed using elevated plus maze, light-dark box, and open field tests between post-HLIR days 16 and 26. Sucrose preference and splash tests assessed depression-related behaviour between post-HLIR days 17 and 20. Quantification of endocannabinoids (2-AG and AEA) and N-acylethanolamines (PEA and OEA) in brain tissue was carried out by LC-MS/MS.

There was no effect of HLIR injury on anxiety- or depression-related behaviour. Female HLIR animals reared for longer than male HLIR animals in the open field test. Lower levels of 2-AG were found in the amygdala of female HLIR animals compared to female shams, with no differences in AEA, PEA or OEA levels. No differences existed in levels of analysed endocannabinoids or N-acylethanolamines in the hippocampus or striatum.

These results indicate sex differences in locomotor activity and the ECS in discrete brain regions following HLIR injury. Further work is required to determine the implications of reduced 2-AG levels in the amygdala of female HLIR rats.

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Poster Presentations

Dr Ruadhan O Laoi ,Trinity College Dublin

"Emergency Department Presentations of Non-Fatal Opioid Overdoses in Dublin, Ireland: A Descriptive Analysis and Examination of 5-Year Mortality Rates"

Authors: Ruadhan O Laoi, James Coulson

Aim: This study aimed to describe the characteristics of non-fatal opioid overdoses presenting to the Emergency Department of a large Irish city-centre hospital in 2014 and the subsequent 5-year mortality rate of these patients.

Methods: Emergency Department presentations with triage categories related to poisonings or self-harm from 01 January to 31 December 2014 were reviewed. Non-fatal opioid overdoses were further examined via retrospective chart review and subsequently descriptively analysed. These cases were then correlated with the National Drug Related Deaths Index from 2015-2019 inclusive to ascertain their 5-year mortality rates.

Main Findings: 278 presentations of non-fatal opioid overdose in 188 patients were identified. The cohort was predominantly male (71%) with a median age of 33 years. The most common presentation of a non-fatal opioid overdose was the intravenous use of heroin without intent of self-harm. Following discharge from the Emergency Department, more than 1 in 7 patients (15.9%) were dead within 5 years at a median age of 38.5 years. The cause of death was due to repeat poisoning in 75% of cases, all of whom were positive for opioids on toxicology and most of whom (90%) had polysubstance detection.

Conclusion: There was a high 5 year-mortality in patients discharged from hospital following a non-fatal opioid overdose. Most notable is the young age of this cohort at presentation and subsequent death. This medical presentation could be utilised to instigate evidence-based interventions that would have a meaningful impact on their opioid use disorder and future risk of harm.

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Poster Presentations

Cillian Power , Department of Pharmacology and Therapeutics, University College Cork

"Investigating the role of NMDA receptor activation in calpain-mediated tau proteolysis: Relevance to Alzheimer's disease"

Authors: Cillian Power, Anirudh V. Jaisimha, Nuala Fitzgibbon, Caoimhe O'Leary, Léa Vinel Djamila Chabane, Anagha Jaggannathan, Mengxi Li, Ellen McClearn, Cora O'Neill, Barry Boland

Alzheimer's disease (AD) is primarily characterized by two major neuropathological hallmarks: amyloid plaques made of insoluble aggregates of amyloid-beta-protein (Aβ) and neurofibrillary tangles (NFTs) that consist of hyperphosphorylated and truncated forms of tau protein. Calpain, a calcium-dependent protease, cleaves tau at different sites, producing various fragments found in NFTs. In this study, we identified mid-range truncated tau fragments (22-50kDa) in early pre-tangle Braak stages of Alzheimer's disease in post-mortem human brain tissue. These fragments were also generated in mature rat primary cortical neuron cultures (14 days in vitro, DIV14)), subjected to simultaneous treatment with glutamate (100uM) and zinc (50uM) over a 6-hour period.

Our investigation suggests that the activation of N-methyl-D-aspartate receptors (NMDARs), which leads to an increased influx of calcium into neurons, triggers calpain-mediated tau cleavage, leading to the formation of these fragments in the initial stages of Alzheimer's disease. The inhibition of calpain using two calpain inhibitors, namely calpeptin (10uM) and ALLN (10uM), reduced the generation of truncated tau fragments associated with excitotoxic stress caused by glutamate (100uM, 6hr), a full media change (6hr), and the NMDA glutamate receptor agonist, NMDA (10uM, 6hr). Notably, inhibition of NMDARs with the specific antagonist, DAPV (10uM, 6hr), was more effective than calpain inhibition in curbing truncated tau generation.

These findings imply that dysfunctional activation of NMDARs could be an early pathological change in the brains of individuals affected by Alzheimer's disease. Furthermore, the capacity of zinc to non-competitively counteract NMDARs may potentially play a role in modulating the progression of Alzheimer's disease.

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Poster Presentations

Aisling Heeran ,Trinity Translational Medicine Institute, Trinity College Dublin

"The role of FKBPL in the Barrett's Oesophagus to Oesophageal Adenocarcinoma disease progression pathway"

Authors:Aisling Heeran, Claudine Duggan, Gillian Moore, Muhammed Waleed Baig, Cian Gargan, Marina Zaki, Meghana Menon, Niamh O'Connor, Christine Butler, Anna Bogdanska, Fiona O'Connell, Marie O'Brien, Sinéad King, Dermot O'Toole, Narayanasamy Ravi, John V. Reynolds, Tracy Robson, Jacintha O'Sullivan

Oesophageal adenocarcinoma (OAC) has a dismal 5-year survival. Barrett's oesophagus (BO) is a preneoplastic condition increasing the risk of OAC. FKBPL is an angiogenesis-related protein with anti-tumour functions and prognostic significance in breast and ovarian cancer. FKBPL-based therapeutics show clinical promise, with one such peptide having successfully completed a phase Ia clinical trial. We hypothesised that FKBPL levels would be reduced across the BO to OAC disease progression.

Methods

We utilised ex vivo human explants from BO and upper GI cancer patients to screen for FKBPL at the mRNA and protein level. We assessed FKBPL levels in the serum of BO and OAC patients and correlated results with clinical parameters.

Results

FKBPL mRNA was significantly higher in normal oesophageal ($p<0.001$) and OAC tissue ($p<0.01$)compared to BO tissue. FKBPL secretion was significantly higher from BO ($p<0.0001$) and upper GI cancer tissue ($p<0.01$) compared to normal tissue. There was no significant difference in the levels of secreted FKBPL between GI cancer tissue and BO tissue. Immunohistochemistry revealed higher expression of FKBPL in the stroma of BO and OAC tissue compared to normal tissue and higher expression of FKBPL in the epithelium of BO compared to normal adjacent tissue in OAC patients. FKBPL levels were significantly higher in serum of BO patients with more advanced disease.

Conclusion

Increased FKBPL may play an oncogenic role in the BO to OAC pathogenesis. Investigation into the functional role of FKBPL in the BO to OAC disease progression may identify potential novel therapeutic targets

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Poster Presentations

Bradan Miller , University College Dublin

"Finely modulating the human macrophage phenotype: A rationale for a pro- resolving approach combined with gold-standard therapy in atherosclerosis"

Authors: Bradan Miller, Monica DeGartano

Introduction

Atherosclerosis is a progressive, multi-factorial, inflammatory and dyslipidaemic disease characterised by the build-up of a plaque, via accumulation of lipid-laden foam cells and cell debris. An imbalanced lipid metabolism and failure to attenuate the inflammation attributes to disease progression. The lack of a therapeutic capable of tackling the associated residual inflammatory risk is an unaddressed need. A key aspect to the progression of this disease is the monocyte-macrophage-foam cell axis, wherein monocytes are activated and differentiated into macrophages for the phagocytosis of infiltrated oxidised low-density lipoprotein (ox-LDL), generating foam cells which contribute to the development of an atheroma (atherosclerotic plaque). Chronic inflammation drives the continuing phagocytosis of ox-LDL and subsequently progresses the disease state.

Aim

We aim to generate, observe, and intervene on a novel in vitro cell model of athero-genesis, specifically monitoring the critical monocyte-macrophage-foam cell axis, in order to identify novel 'druggable targets' in the Resolution of Atherosclerosis.

Methods

THP-1 monocytes were cultured and differentiated in the presence of differentiating agents (50 nM PMA, 100 ng/ml M-CSF, 100 ng/ml GM-CSF, or Vehicle) for 78 hrs, and then being subsequently polarised with key cytokines (20 ng/ml IL-1β, IL-4, IL-10 or TRAIL) for 48 hrs, to develop an array of macrophages phenotypes, characterised through transcriptomic analyses. Following successful differentiation into various phenotypes, 50 g/ml of ox-LDL was added to the model for the formation of foam cells, which was monitored using Sartorius Incucyte technology.

Results

Through the use of a combination of cell imaging and single gene analysis (by RT-PCR) of the individually treated cells, a significant upregulation of key markers of differentiation (CD14 and CD68) was observed, confirming monocyte-macrophage. Furthermore, analysis of notable genes associated with anti-inflammatory, pro-inflammatory, or pro-resolving activity was performed to identify a plethora of macrophage phenotypes generated as a result of the aforementioned polarising agents. An accumulative transcriptomic heat-map was generated to identify the various phenotypes as the cells progressed from monocytes to macrophages.

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Foam cell generation was assessed via further imaging (live imaging with the incucyte) and gene expression analysis. These data allowed for the determination of macrophage susceptibility to ox-LDL uptake via regulation of key transporter proteins and scavenging receptors, whilst efficiency of uptake was monitored via fluorescently tagged ox-LDL.
Significant uptake of ox-LDL was observed in TNF-treated cells when compared to a vehicle- treated subset, highlighting the role of the pro-inflammatory macrophage phenotype (M1) in the generation of foam cells, and the subsequent accumulation of these cells for the formation of an atheroma.

Summary

We have successfully set-up an in vitro model of atherosclerosis pathogenesis along the monocyte-macrophage-foam cell axis. By monitoring the transcribed/secreted levels of key differentiating factors, cytokines, and transporters, we have generated a heat-map exemplifying the macrophage sub-types generated, contributing to the plasticity of our model. To finalise validation of the model, similar analyses allowed for the confirmation of foam cell generation in the cell model, completing the elucidation of the differentiation-axis of these key cells during atherosclerosis pathogenesis.

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Poster Presentations

Mai Alkurashi , School of Biochemistry & Cell Biology, Biosciences Institute, University College Cork

"Inhibition of the endosomal recycling pathway overcomes resistance to tyrosine kinase inhibitors in Glioblastoma Multiforme brain cancer"

Authors:Mai H. Alkurashi and Andrew J. Lindsay

Glioblastoma Multiforme (GBM) is a highly aggressive primary brain cancer, classified by the WHO as a grade IV. The standard therapy includes resection, radiotherapy, and chemotherapy. These conventional therapies yield a median overall survival (OS) rate of 15 to 18 months, with approximately 20 to 25% of cases reaching 2 years. Consequently, there is a clear unmet need to develop novel therapies that can improve the OS of GBM patients [1].

The epidermal growth factor receptor (EGFR) is one of the receptor tyrosine kinase (RTK) that are linked to poor prognosis, and is amplified or mutated in 40–60% of primary GBM. Clinical trials targeting EGFR with tyrosine kinase inhibitors (TKIs) have yielded disappointing results. Previous work from our lab demonstrated endosomal recycling inhibition (ERI) reduced the total protein levels and downstream signalling of several clinically relevant RTKs in breast and lung cancer [2]. Therefore, we sought to modulate the levels of EGFR and its downstream signalling pathways by disrupting their intracellular trafficking.

We observed strong synergy between ERI and both TKIs in cell proliferation, clonogenic and 3D spheroid assays. Further, we used Western blots to analyse the effect of the drugs and drug combinations on the activation and total protein levels of the RTKs and their downstream signalling pathways.

These findings suggest that the ER pathway could be a valuable and overlooked target for innovative treatments for cancer, and ERIs have the potential to enhance the effectiveness of targeted therapies that are currently employed in clinical practice.

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Poster Presentations

Rasha Alshaikh , School of Pharmacy, University College Cork

"Siponimod as a Novel Angiogenesis Inhibitor: Design of an Electrospun Sustained Release Implant for Intravitreal Injection"

Authors: Rasha A. Alshaikh, Christian Waeber, Katie B. Ryan

Pathological angiogenesis plays a pivotal role in several ocular diseases, leading to treatable or preventable vision impairment, such as diabetic retinopathy and wet-AMD. Currently, approved treatments for these conditions involve intravitreal injections of anti-VEGF biologics to impede angiogenesis. However, these agents are associated with issues like drug resistance, limited stability, and a short ocular half-life. Consequently, there is a pressing need to identify alternative pharmacological targets for inhibiting ocular angiogenesis and to develop sustained-release delivery options.

Siponimod shows promise as an alternative treatment that operates by modulating sphingosine-1-phosphate receptor-1 to inhibit critical steps in angiogenesis and enhance endothelial barrier function. In this study, we designed an injectable electrospun implant containing siponimod for intravitreal injection. The implant was able to produce sustained drug release, reducing the need for frequent injections. The implant was created by electrospinning an 8% w/w solution of siponimod within a PLGA 85:15 solution. The electrospun siponimod implants exhibited a dense micro-fibrous structure, with no alteration in the physical properties of the PLGA matrix post-electrospinning. The release profile from the electrospun implant demonstrated a consistent, sustained release of the drug for at least 90 days. After this period, siponimod remained stable within the implant, and the released drug effectively inhibited the migration of endothelial cells. The produced implant can offer an alternative treatment with a less frequent administration regimen and a novel mechanism of action for ocular angiogenesis.

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Poster Presentations

Bhavya Mugawar , School of Medicine, Trinity College Dublin

"Statin intolerance and its stakeholders"

Authors: Bhavya Mugawar, Cormac Kennedy, Patricia O'Connor

Background: Hypercholesterolemia is a well-described risk factor for atherosclerotic cardiovascular disease (ASCVD) .Statins remain the cornerstone of therapy. Statin intolerance (SI) particularly statin associated muscle symptoms (SAMs) and inappropriate stopping of treatment is associated with increased cardiovascular risk. A significant proportion of reported SAMs relates to expectation of side effects and can be termed the 'negative drucebo effect'. Patients should be educated about SI, the negative drucebo effect, in addition to the benefits of adherence to the therapy when first prescribed a statin. A diagnosis and management plan for SAMS which can be used in primary care is important.

Aims: To run a pilot with respect to education of patients attending the lipid clinic at St. James's Hospital (SJH), Dublin, about SI and the 'negative drucebo effect' and to develop a SAMs diagnosis and management plan.

Methods: This pilot project was carried out over a two-week period. A patient information leaflet (PIL) was developed explaining the concepts of SI and the 'negative drucebo effect'.. In person feedback was obtained from patients, following distribution of drafts of the PIL to patients during their lipid clinic appointments. The project was explained to them and they had the chance to read the PIL and give feedback. A SAMs diagnosis and management plan was also designed based on existing guidelines.

Conclusion: The PIL was well received by the 6 patients asked. A SAMs diagnosis and management plan has been developed amid a future pilot project using this is planned. Challenges and learning point encountered while making a PIL, included explaining the information and current research in a patient friendly manner. Similarly, designing the SAMs diagnosis and management plan was challenging with respect to identification of the clinical decision points required to branch the pathway in different directions.

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Poster Presentations

Emma Cagney , Mercy University Hospital, University College Cork

"An Audit of the Use of the Direct Oral Anticoagulants (DOACs) "

Authors: Emma Cagney, Aoife Fleming, Ciaran Halleran

The problem being addressed in the study

DOACs are a novel group of medications. They are high risk medications and incorrect use can result in significant patient morbidity and mortality.

·To audit use of the DOACs in terms of dose and appropriateness, then determining the level of pharmacist intervention required.

·To establish which patient specific factors are associated with inappropriate use.

·To assess transitions to/from other anticoagulants in terms of safety.

How the study was performed

Over a six month period, inpatient's DOAC prescription were audited. Specific data was gathered to determine the appropriateness of each prescription. Statistical analysis was then carried out to establish the rate of inappropriate use and the impact of any patient parameters on this.

The salient results

159 patients were audited. Inappropriate DOAC prescriptions requiring pharmacist intervention were identified in 92 patients (57.9%). This was at the upper end of what had been reported in the literature 7%-60%. Apixaban was most associated with inappropriate use ($p=0.038$), with statistical significance demonstrated with the 2.5mg dose. Patient factors associated with inappropriate use were male gender ($p=0.003$) and having ≥ 5 co-morbidities ($p=0.018$).

74.5% of transitions between DOACs and other anticoagulants were inappropriately managed.

What the authors conclude

A high level of inappropriate use of DOACs was identified, requiring significant pharmacist intervention. The need for more prescriber education and support was acknowledged. Such supports may include future work such as smart phone applications to calculate appropriate doses and guide timings for transitions between anticoagulants. To prioritize patient safety there is need for greater pharmacist involvement in DOAC prescribing

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Poster Presentations

Edel O'Dea , Wellcome HRB Clinical Research Facility, St. James's Hospital

"Delivering Bacteriophage Therapy – A Collaborative Approach "

Authors: Keelan McManus, Siobhan Berry, Susan Clarke, Pradash Madhavan, Niall Mc Eniff, Concepta Merry, Edel O Dea, Nicholas Power, Derval Reidy, Martina Hennessy

Background:

Bacteriophage ('Phage') therapy is a promising anti-bacterial treatment which holds potential as a novel approach in treating multidrug resistant (MDR) infections. However, its integration and implementation in an acute hospital setting presents a unique set of challenges. This presentation examines such challenges offering insights into the complexities faced during the delivery of Phage therapy. It explores the critical role of the multi-disciplinary team (MDT) in ensuring the safe and effective treatment with Phage from procurement and quality control to storage, preparation and administration. It will discuss the regulatory landscape associated with this pioneering treatment modality, emphasising the necessity for standardised policies and procedures.

Aim and Objectives:

The objective was to develop and implement a comprehensive process for the procurement, handling, preparation and delivery of Phage therapy.

Methods:

A multidisciplinary team was assembled and regular meetings were scheduled. The MDT liaised with the Health Product Regulatory Agency (HPRA) to determine the legislative parameters for bringing bacteriophage therapy to our site. We engaged international experts to learn from their experiences and to ensure best practice would be followed throughout the entire process.

A review of the literature was carried out and a risk assessment was undertaken. A detailed procedure was developed for the ordering, receipt, storage, preparation and administration of bacteriophage. Other key stakeholders were identified and an extensive communication and training plan was implemented to ensure all relevant staff were adequately trained and roles and responsibilities were understood. This also involved the development of a patient information leaflet and a staff information leaflet.

Results

A procedure was developed and agreed which ensured the safe delivery of Phage therapy from procurement to administration to post treatment follow-up. This is an important step in establishing proof of concept and potentially making this treatment available to a wider patient cohort in the future. Phage therapy was delivered in St James's Hospital for the first time allowing a patient access to this personalised care over a 14-day treatment period comprising of 15 individual Phage products.

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Conclusions and future work.

A collaboration between the Wellcome HRB Clinical Research Facility and St James's Hospital demonstrated that Phage therapy can be safely delivered at this institution. Further work is required to explore how this therapy can be made available to more patients. This would require a multi-disciplinary approach to scale up the service based on the existing model. Key aspects to explore for expansion of the service would include resources (such as cleanroom facilities and staff), establishing ongoing Phage procurement supply and patient selection criteria.

Acknowledgements

The authors acknowledge the advice and assistance provided by the Queen Astrid Military Hospital Belgium, Josh Jones NHS Clinical Phage Specialist and University of California San Diego

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Poster Presentations

Edmond Morrisey, Mercy University Hospital, Department of Medicine, University College Cork

"Sustained Success and Savings from Switching to Subcutaneous Vedolizumab "

Authors: E Morrissey, R Varley, K Sugrue, C O Sullivan, S Ghosh, D Sheehan, C Moran, M Buckley, J McCarthy

(I)Introduction

Vedolizumab (VDZ) is an important treatment in the management of inflammatory bowel disease (IBD). It has traditionally been administered as an intravenous(IV) infusion but is now available as a subcutaneous (SC) injection. There is a lack of real world data around SC VDZ use and drug levels. This study assesses SC vedolizumab drug levels and efficacy. It also assesses savings associated with SC use.

(II) Methods

VDZ trough levels from when the patient was receiving IV VDZ were compared to the most recent trough level after switching to SC VDZ. Response to SC VDZ was assessed using faecal calprotectin. A subset of patients completed a questionnaire about attitudes to SC therapy. The financial cost and time cost of providing IV VDZ to each patient was assessed.

(III) Results

95 patients were eligible for inclusion. Average age was 36 (18-78). 51 (54%) had ulcerative colitis (UC), 44 (46%) had Crohn's disease (CD). VDZ trough levels improved by an average of 11.51ug/mL (54%) after switching to SC ($P<0.05$). Faecal calprotectin fell by an average of 83.49ug/g (-18.5%) after switching to SC formulation ($P<0.05$). In a subset of patients surveyed (n=10) 90% preferred SC to IV VDZ. Our hospital saves 2.13 million euro per year due to the SC switch of the 95 patients included in the study. Our department saves 614 (20.5% of total capacity) infusion suite appointments per year due to the SC VDZ switch.

(IV) Conclusions

Switching from IV to SC VDZ is associated with improved VDZ levels and improved faecal calprotectin levels. Most patients expressed a preference for SC therapy. Switching from IV to SC VDZ saves significant amounts of time and money for our department.

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Poster Presentations

Jonathan Costello , School of Medicine, University of Galway

"Differential effects of TLR3 activation on social behaviour and prefrontal cortical inflammatory gene expression in the valproic acid model of autism: a role for the endocannabinoid system? "

Authors: Jonathan A Costello, Aoife M Thornton, David P Finn & Michelle Roche

Introduction: Autism is associated with immune alterations and neuroinflammation(1). Increasing endocannabinoid tone attenuates autism-related behavioural changes in models(2) and modulates TLR-induced neuro-immune responses(3). This study examined TLR3 activation, in the presence or absence of a FAAH inhibitor, on behaviour, neuroinflammatory gene expression and endocannabinoid levels, in a preclinical rodent model of autism.

Methods: Female Sprague-Dawley rats prenatally exposed to saline or VPA received 1) polyI:C (3mg/kg i.p.) or saline vehicle and were euthanised 4h later OR 2) the FAAH inhibitor PF3845 (10mg/kg i.p.) or vehicle, prior to polyI:C, and underwent nociceptive and social behaviour testing 24h later before euthanasia. The PFC from both cohorts was assessed for endocannabinoid and N-acylethanolamines levels using LC-MS/MS and inflammatory gene expression using RT-qPCR.

Results: PFC inflammatory gene expression was increased in saline-exposed rats at 4h & 24h post polyI:C. In comparison, in VPA-exposed rats, polyI:C-induced increases in IL-1β and CCL2 expression were blunted at 4h, while inflammatory gene expression returned to baseline levels at 24h. PFC 2-AG levels were reduced in saline-exposed poly I:C-treated and VPA-exposed vehicle-treated rats, when compared to saline-exposed vehicle-treated counterparts. PF3845 increased N-acylethanolamine levels in saline- and VPA-exposed rats, an effect associated with blunted poly I:C-induced inflammatory gene expression in saline-exposed rats only. Nociceptive responding did not differ between the groups, however polyI:C reduced social novelty preference of VPA-, but not saline,-exposed rats, an effect unaltered by PF3845.

Conclusion: VPA-exposed female rats display differential behavioural and neuroimmune responses to a viral immune challenge, which were unaltered by FAAH inhibition

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Poster Presentations

Suraj N. Chembukavu ,School of Biochemistry & Cell Biology, Biosciences Institute, University College Cork

"Evaluating the impact of endosomal recycling inhibitors on therapy-induced senescent breast cancer cells "

Authors:Suraj N. Chembukavu and Andrew J. Lindsay

Extended treatment with chemo- and targeted- therapies induces a quiescent state in breast cancer cells, characterised by differences in physiological and morphological properties. Such cells are said to enter therapy-induced senescence (TIS).

This research investigates the role that membrane trafficking pathways play in inducing and maintaining TIS. We have previously reported that the endosomal recycling pathway is upregulated in HER2-positive breast cancer cells induced to enter senescence through long-term treatment with HER2-targeting therapies. We reported that these cells are more sensitive to the cytotoxic effects of small molecule endosomal recycling inhibitors (ERIs) than non-senescent cells. We hypothesised that cells that have entered TIS have a hyperactive endosomal recycling pathway and are thus vulnerable to ERIs.

To investigate whether this is exclusive only to senescent HER2+ breast cancer cells, we induced TIS in cell lines representing several breast cancer subtypes (HER2+, hormone receptor positive (HR+), and triple-negative breast cancer). Senescence was confirmed by measuring lysosome content and SA-β-galactosidase activity. The cytotoxicity of ERIs in the parental (non-senescent) and senescent cells was compared.

We found that senescent HER2+ breast cells were consistently two-fold more sensitive to an ERI called primaquine. We found that HR+, but not TNBC, cells were also hypersensitive to ERIs. These findings indicate that inhibition of the endosomal recycling pathway may be a useful strategy to kill breast cancers that have developed resistance to HER2-targeting therapies. We are currently investigating the molecular mechanism of action of ERIs by comparing the levels of TIS markers in untreated and ERI-treated cells.

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Poster Presentations

Aron Barron ,Department of Pharmacology and Therapeutics, University College Cork

"Exposure to maternal pre-eclampsia serum increases neurite growth and mitochondrial function through an IL-6-dependent mechanism in an in vitro model of neurodevelopment."

Authors: Aaron Barron, Samprikta Manna, Colm McElwain, Andrea Musumeci, Fergus McCarthy , Gerard W. O'Keeffe, Cathal M. McCarthy

Introduction: Prenatal exposure to the common hypertensive disorder of pregnancy, pre-eclampsia (PE), increases the risk of neurodevelopmental disorders in exposed offspring. However, the cellular and molecular basis of this increased risk are currently unknown.

Objective: To determine the effects of exposure to maternal serum from women with PE on an in vitro model of neurodevelopment, and investigate potential molecular mechanisms involved.

Methods: Human neuroblastoma SH-SY5Y cells were neuronally-differentiated with retinoic acid and brain-derived neurotrophic factor. We examined the effects of maternal serum from women with PE or a healthy uncomplicated pregnancy on the survival, neurite growth and mitochondrial function of differentiated SH-SY5Y cells. Following this, we investigated the pleiotropic cytokine IL-6 as a potential mechanism of any observed effects.

Results: Cells exposed to PE serum exhibited increased neurite growth and mitochondrial respiration, two important neurodevelopmental parameters, compared to those treated with control serum ($n=10\text{-}13$, $p < 0.05$). Levels of IL-6 were significantly elevated in maternal PE sera and placental explant supernatants ($n=4\text{-}5$, $p < 0.05$), and in agreement with this, cells exposed to PE serum had increased phospho-STAT3, a key intracellular mediator of IL-6 signalling ($n=4$, $p < 0.05$). Furthermore, neutralizing IL-6 with a function-blocking antibody prevented the effects of PE serum on neurite growth ($n=5$, $p < 0.05$), whereas exposure to IL-6 induced a similar phenotype to PE serum.

Conclusions: Collectively these data show that PE results in elevated serum levels of maternal IL-6, which mediates an increase in neurite growth and mitochondrial function in differentiated SH-SY5Y cells.

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Poster Presentations

Ye Yang ,School of Pharmacy and Biomolecular Sciences (PBS), Royal College of Surgeons in Ireland

"A Role for Astrocytes in the Regulation of Platelet Function in Thrombosis and Stroke."

Authors: Ye Yang, Huiling Zhang, Niamh Moran

Background: Astrocyte activation contributes to blood-brain barrier breakdown in thrombotic stroke. The subsequent interaction between astrocytes1 and platelet plays an essential role in pathological thrombosis, but the nature of this interaction is not yet understood.

Objective: To determine if the releaseate from stress-activated astrocytes can modify platelet function. Methods: Platelet rich plasma (PRP) from healthy volunteers was prepared as previously described2. Threshold agonist doses (TRAP or ADP), that induced <20% of maximal platelet aggregation and secretion responses were identified. Human Astrocytic (HA; Sciencell, Carlsbad, CA) cells were stressed for 6 hours in (1) normoxic, serum-deprived DMEM or (2) hypobaric, oxygen/glucose-deprived (OGD) medium. Conditioned medium (CM) was concentrated using Amicon 30kDa or 50kDa MWCO filters. PRP was pre-treated with CM or corresponding controls, before being challenged with threshold doses of agonists.

Results: Alone, HA-CM has no effect on platelet aggregation or secretion. Compared to DMEM, serum-deprived HA-CM (>50kd and >30kd) substantially enhanced platelet aggregation induced by threshold doses of TRAP (P=0.033; P=0.0421, n=6) and ADP (P=0.021, P=0.038, n=4); Similarly, OGD-CM (>50kd and >30kd) significantly enhanced platelet aggregation induced by these agonists (TRAP:P=0.012; P=0.025, n=3; ADP:P=0.043; p=0.036, n=3); HA-CM equally enhances the magnitude of dense-granule secretion from platelets. TNF-alpha cytokine was not responsible for the platelet-enhancing effect, as determined by ELISA.

Conclusion: Protein-releasates from physiologically-stressed HA cells significantly enhance platelet aggregation and secretion responses activated by sub-threshold doses of agonists. Understanding the nature of this CM component may give insights into why cerebral thrombi are resistant to lysis by thrombolytic agents3

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Poster Presentations

Monica DeGaetano , University College Dublin

"Characterization of the human macrophage spectrum:
a novel approach to identify novel drug targets and biomarkers in atherosclerosis"

Authors: Monica DeGaetano

Clinical challenge Atherosclerosis, the underlying cause of heart attack and stroke, is an extremely complex and multifactorial disease. It is characterised by the progressive accumulation of lipids and cell debris, confined in an atherosclerotic plaque, which narrows the lumen of the major arteries.

Therapeutic unmet need Atherosclerosis, essentially due to a sustained status of dyslipidaemia and low-grade inflammation, can remain sub-clinical for decades during the lifetime of an individual, until major cardiovascular events occurs. Therefore, it is crucial to diagnose the disease at early stages of progression, in order to intervene more efficiently with a more personalised therapy. Currently, we are still lacking specific biomarkers for determining the building-up of atherosclerotic plaques at an initial stadium.

Cellular and Molecular Background A key cellular player during the progression of the disease is the monocyte-macrophage-foam cell axis: once endothelial damage activates monocytes, these are recruited to the inflammatory sites, extravasate and transform into a macrophage, which engulfs lipids and cell debris, turning into a foam cells, thus forming the necrotic core of the plaque.
Aim of the study To develop a robust in vitro model of 'early-to-late' atherogenesis along the monocyte/macrophage axis, in order to identify novel drivers of the resolution of inflammation in the context of atherosclerosis, which could be targeted by novel drug candidates during the resolution phase in athero-regression.

Methods By using a THP-1 monocytic cell line, sub-cultured in a 24-well-plate miniaturised-format, various differentiating agents (50 nM PMA; 100 ng/ml M-CSF or GM-CSF) for 72hrs, followed by exposure to a series of polarising agents (20 ng/ml IL-1β, IL-4, IL-10 or TRAIL) for 48hrs, were used to induce a plethora of macrophage sub-phenotypes. After a week of differentiation/polarization, the mature macrophage was exposed to an hyper-lipidaemic stimulus (specifically, 50 □g/ml of ox-LDL, for 4 hrs) to induce foam cell formation, upon lipid uptake

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The changes in cell morphology, occurring during the transformation of naïve monocytes into mature macrophage and ultimately foam cell formation, were closely monitor via imaging, by using both Transmission Light Microscopy (TLM) and digital imaging (thanks to the Sartorious Incucyte technology, capturing images at 1-hour interval over days at 37°C). Single gene analysis was performed via RT-PCR by measuring mRNA expression of key markers along the monocytic-derived lineage, including: CD-14 and CD-68 (specifically, monocyte and macrophage pan- markers); TNF and CD163 (respectively, M1 and M2-phenotype markers); MCP-1 and MIP-1a (pivotal monocyte/macrophage chemo-attractants); IL-18/IL-1β/IL-6 (NLRP3 inflammasome axis); TRAIL (key counterpart of NLRP3); CD36 and SRA1 (influx proteins for lipid-uptake); ABC-A1 and ABC-G1 (efflux proteins in foam cells).
During macrophage differentiation, activation of the NLRP3 inflammasome pathway and its (TRAIL-driven) counterpart was also measured at protein level by chemo-luminescence-based multiplex ELISA. Foam cell formation was also validated through a lipid uptake assay, by measuring the % of fluorescently- tagged Dil-ox-LDL engulfed by the macrophage, via a live-monitoring Incucyte technology).Statistical analysis was performed on N=3±SEM (normalized to GAPDH, for gene analysis; and to cell density for ELISA and uptake assay). F-tests was used to inform on the appropriate (2-tailed) T-tests (p-val < 0.05).

ResultsThe in vitro model of macrophage maturation and foam cells formation was successfully set up: the differentiation phases were tracked by imaging, capturing in real-time the rounded shape of naïve monocytes, followed by a spreading in morphology after 3 days, ultimately displaying a 'bubbly' foamy appearance, subsequent to lipid uptake, after 7 days. The plethora of macrophage sub-phenotypes were monitored at gene level, via modulation of the key cell markers, thus generating 'signature' heat-maps for each specific sub-phenotype. In particular, culturing of unstimulated-naïve monocytes led to an MF0-macrophage after 3-days and to an inflammatory MF1-macrophage, after 6-days. If a strong differentiating agent, such as PMA, was added at day-3, transformation into the MF1-macrophage was accelerated. This MF1-phenotype became more pronounced at day-6, but pro-resolving or regulatory agents, such as TRAIL or IL-10, pushed back the inflammatory macrophage towards the MF0-quiescent state. Moreover, mature macrophages significantly released the pivotal IL-18 inflammasome cytokine even after 48 hours from M1-polarising stimuli, whilst the release of downstream effectors of the same pathway was switched off at that time.Foam cell formation was successfully measured via upregulation of scavenger receptors genes(in particular, CD36 was significantly upregulated after exposure to ox-LDL), as well as by a significant engulfment of fluorescently-labelled lipids, measured at the Incucyte, as a ratio between lipid-loaded cells/cell density.

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Poster Presentations

Lauren Fernandes , University of Limerick

"The Effect Of An Education Session On Medication Documentation Quality In A Model 2 Irish Hospital: A Closed Loop Audit"

Authors: Lauren Fernandes, Ethar Eltayeb, Margaret O'Connor, Marwa Mustafa, Ahmed Gabr, Wen Jie Koay, Abdirahman Mohamed, Edmond Morrissey, Zaheer Iqbal

Background: Medication errors are associated with significant morbidity, mortality and cost.

The three key priority areas identified by a WHO initiativeare high-risk situations (e.g. older patients), polypharmacy and transitions of care; all of which are pertinent to our setting. The aim of our study was to examine the quality of documentation of prescribing and transcribing in an Irish model 2 hospital.

Method: A retrospective chart review of 100 hospital inpatients was conducted (50 at initial audit stage, and a further 50 during reaudit). An adapted version of an existing patient safety instrument was used for data extraction. A prescribing education session was held with NCHDs and reaudit was performed 10 weeks later.

Results: The average age within our patient cohort was 76. The rates of polypharmacy and hyperpolypharmacy were 76% and 46% respectively. Completeness of prescribing for PRN medications (individual & maximum dose, indication) and discontinuations (dated, initialled, reason indicated) were only 35% and 21% initially; with a respective decline to 33% and increase to 31%. Overall completeness of both prescribing of regular medications and transcribing were high for both groups with a slight improvement post-intervention. Allergy status documentation improved from 90% to 100%, while documentation of nature of reaction declined from 43% to 31%.

Conclusion: Our findings did not demonstrate a convincing or consistent improvement in prescribing or transcribing quality following an education session with NCHDs. This is consistent with a recent Cochrane review. The best evidence for addressing medication errors appears to be for computerised physician order entry systems, which minimise room for human error

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Poster Presentations

Donatas Galickas, University Hospital Limerick

"Clinical Audit of Medical Reconciliation in an Acute Medical Unit in a Model 4 Hospital"

Authors: Donatas Galickas, Ciara McNulty, Ida Carroll, Edmond Morrissey, Marwa Mustafa, Anne Harnett, Ahmed Gabr, Nora Cunningham, Margaret O Connor

Background Medication reconciliation was identified as an essential tool to reducing patient harm by the World Health Organization and was included in the "Action of Patient Safety 5S programme". The Institute of Medicine's Preventing Medication Errors report stated that the average hospitalised patient is subject to at least one medication error per day. Previous audits indicated error rates of 54% in medication documentation following acute hospital admission. Most medication errors identified occurred in those with polypharmacy and multimorbidity. Implementation of medication reconciliation for acute admissions was the recommended action.

Aims

This audit aimed to establish the rate of medication reconciliation and polypharmacy in patients >65 presenting to the Acute Medical Unit (AMU).

Methods

Eligible patients were identified using a ward census. Inclusion criteria: age over 65, admission to the AMU during the audit period (four midweek days between 13-20th October 2023). Patients transferred to another ward or discharged were excluded. Patient data was collected from medical notes and analysed using Microsoft Excel.

Results

A total of 17 patients were eligible for inclusion. 11/17 (65%) were prescribed more than 5 medicines. 10/17 (59%) patients had >5 co-morbidities documented. 13/17 (76%) had at least one risk factor for delirium. No patients had a medication reconciliation documented and none were screened for delirium.

Conclusions

Polypharmacy and multimorbidity were common, however medication reconciliation was not routine in the AMU. Resourcing of medication reconciliation processes in AMU units is an urgent service imperative. Criteria could be developed to target medication reconciliation towards those at highest risk.

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Poster Presentations

David Walley , School of Medicine, University College Dublin

"AAV8-PCSK9 induced Hypercholesterolaemia and Atherosclerosis in Mucosal Mast Cell COX-2 Deficient Mice."

Authors: David Walley , Soon Yew Tang , Ronan Lordan, Ujjalkumar Das, Robin Joshi, Carsten Skarke, Garret A. FitzGerald

Non-steroidal anti-inflammatory drugs (NSAIDs) selective for cyclooxygenase-2 (COX-2) carry cardiovascular risks. Mast cells contribute to atherosclerosis by accumulating in perivascular tissue. We investigated the effect of COX-2 deletion (KO) in mucosal mast cells (MMCKO) to determine their role in atherosclerotic burden (AB) in a hypercholesterolaemic murine model.

Chm Cre +/- /COX-2 FF mice were generated as MMCKOs and Cre-negative littermates used as controls. Hypercholesterolaemia was induced using retro-orbital administration of AAV8/Pcsk9 and feeding mice (n = 12) a western diet (WD) for 12 weeks. Non-invasive tail-cuff system or radio-telemetry was used to measure blood pressure (BP), heart rate (HR) and physical activity (PA). Aortae were stained using Sudan-IV and AB was determined by en-face analysis. Liver RNA was extracted and relative gene expression assessed by RT-qPCR. Systemic prostanoid biosynthesis was determined by mass spectrometry. Plasma glucose, LDL, HDL and triglycerides were measured using biochemical assays.

AAV8/Pcsk9 elevated PCSK9 expression and induced hypercholesterolaemia. Urinary prostanoid metabolite profile displayed sex and genotype differences. AB was greater in males; however, no significant differences were observed between controls and KOs in both sexes. BP, HR and PA were not significantly altered. Plasma HDL and LDL levels were significantly elevated after feeding WD but not glucose and triglyceride levels. A larger cohort of mice is needed to be fed WD for longer durations. Higher or additional doses of AAV8/Pcsk9 may be administered to maintain hypercholesterolaemia. Increased understanding of the role of COX-2 in mucosal mast cells in atherosclerosis may help attenuate the risks of chronic NSAID administration.

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Poster Presentations

Caitlin Gibson , University of Limerick

"3-M syndrome – a rare complication of Pembrolizumab"

Authors: Caitlin Gibson, Marwa Mustafa, Wen Jie Koay, Mohanad Abdulrahman, Abdirahman Mohamed, Amro Babiker, Colum Horan, Edmond Morrissey, Margaret O'Connor, Roshni Kalachand, Aoife Leahy, Ahmed Gabr

Background

Immune checkpoint inhibitors (ICIs) are revolutionary pharmacological therapies with wide utilization in various types of cancers. Recent research explored the benefit of Pembrolizumab in metastatic castration-resistant prostate cancer. These drugs have been associated with several immune-related serious adverse events (irAEs).

Case Presentation

We present a case of a 75 year old male diagnosed with metastatic prostate adenocarcinoma, T2bN1M1a (Gleason 7), refractory to traditional hormonal as well as radiation therapies. Following disease progression, he was enrolled in a cancer clinical trial utilizing pembrolizumab and tolerated this initially well. However, treatment was stopped due to the development of progressive severe fatigue, shortness of breath, muscle weakness with progression to respiratory failure requiring intensive care support. He was treated for pneumonitis, complete heart block due to myocarditis with pacemaker insertion as well as myositis. He described ongoing bulbar symptoms including dysphagia, dysarthria, as well as ocular symptoms such as diplopia and ptosis. EMG studies were carried out with confirmatory diagnosis of de-novo Myasthenia Gravis. He was treated initially with high dose intravenous steroids as well as immunoglobulins, plasmapheresis and pyridostigmine with satisfactory response then transitioned to Rituximab as a steroid sparing agent. His disease ultimately progressed and the patient declined any further treatment. He was commenced on a palliative care pathway and died peacefully.

Conclusion

This case highlights a rare irAEs "3-M" syndrome composed of Myositis, Myocarditis, Myasthenia Gravis. Despite the effectiveness of immune checkpoint inhibitors, it is important for clinicians to recognise their rare but serious irAEs where treatment suspension and early institution of high dose steroids may reduce morbidity.

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Poster Presentations

Marwa Mustafa , University of Limerick

"The hazards of Clozapine "

Authors: Marwa Mustafa, Caitlin Gibson, Edmond Morrissey, Wen Jie Koay Nurul Othman, Abdulrahman Mohaned, Donatas Galickas, Nora Cunningham, Ida O Carroll, Margaret O'Connor, Ahmed Gabr

Introduction

Clozapine is a drug associated with multiple adverse effects but has good efficacy for resistant psychosis. While prescribing is within the remit of specialist psychiatry, many patients prescribed clozapine attend hospital under the care of internal medicine physicians, requiring a knowledge of key principles.

Cases

A 34-year-old gentleman with schizoaffective disorder presented with chest pain, elevated troponin, diffuse upsloping ST elevation on ECG and normal ECHO and was diagnosed with myopericarditis, a known adverse effect of clozapine. His psychiatric condition had been only partially responsive to treatment until commencement of clozapine. In view of the potential for cardiomyopathy and worsening myocarditis, the psychiatrist agreed with cessation of clozapine with plans for an alternative along with NSAIDs for pericarditis.

A 68-year old schizophrenic lady presented with falls on clozapine and perindopril for hypertension. Head up tilt testing confirmed a progressive drop in systolic blood pressure (BP) over 3 minutes of 90 mmHg with a 24-hour-ambulatory BP monitor showing 144/89. Perindopril was discontinued. Multidisciplinary discussion with psychiatry was undertaken with a recommendation that clozapine be continued due to difficult to manage psychiatric symptoms. Clozapine binds to multiple receptors. Alpha-1 adrenoreceptor blockade causes autonomic dysfunction and droxidopa (a noradrenaline precursor) was commenced after lack of response to midodrine and fludrocortisone with some amelioration of symtpoms.

A 54-year-old woman was admitted with acute kidney dysfunction due to gastroenteritis. All medications including anti-hypertensive/diabetic drugs and clozapine were held by the admitting doctor. After 24 hours, a medication reconciliation by pharmacy advised to urgently recommence clozapine as prolonged interruption of clozapine would need a lower dose re-initiation regimen.

Conclusion

Drug discontinuation is the usual preference with drug-adverse effect rather than a prescribing cascade to manage adverse effects. With limited options for resistant psychiatric disease, clozapine may need to be continued. Management of clozapine requires multidisciplinary discussion between pharmacy and psychiatry teams with potential for significant drug interactions and adverse effects.

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Poster Presentations

Anne Harnett , University of Limerick

"Medicines expenditure in an Irish acute hospital "

Authors: Anne Harnett, Susan Stack

Which classes impact disproportionately?

Method:
Dispensing data provided by UHL hospital pharmacy for quarter two 2023 are analysed by the AHDMP and sorted into a top 20 ranking by expenditure, each drug being identified by its ATC code. The expenditure per ATC code was calculated as a percentage of the total spend on medicines.

Results:
For Q2 2023:
- Expenditure on top-20 medicines by drug was 56% of the total expenditure on medicine for the Q2 2023.
- Fourteen (14/20) were identified as available from a sole source supplier.
- Seventeen (17/20) were biological medicines of which 13 were monoclonal antibodies, 2 were enzymes, 1 was a hormone and 1 was a decoy receptor.
- Three (3/20) were chemical medicines of which only one was an oral formulation.
- Indications for medicines in the top 20 were cancer, inherited enzyme deficiency, HIV infection, anaemia and inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease and asthma.

Conclusion:
Over half of the annual expenditure on medicines in an acute model 4 hospital is attributable to 20 ATC codes. While the majority of medicinal products are chemicals the majority of expenditure on medicines in acute hospital settings is on biological medicines.

Foot note
AHDMP - Acute Hospital Drug Management Programme

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Poster Presentations

Anne Harnett , University of Limerick

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