Irish Association of Pharmacologists

18th Annual Meeting

Friday, 24th November 2017

Hosted by

School of Medicine, Conway Institute of Biomolecular & Biomedical Research, University College Dublin

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Irish Association of Pharmacologists

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Irish Association of Pharmacologists 18th Annual Meeting 24th November 2017, UCD Conway Institute

Contributor/Event	Title	Time
Meeting	Irish Association of Pharmacologists Annual General Meeting **Members Only**	10.45-11.20
Tea & Coffee Registration	Registration & Tea/Coffee	11.00-11.30
Opening Address	Welcome and Opening Address: Assoc. **Prof Helen Gallagher** - Chair Local Organising Committee/**Prof Thomas Walther** – IAP President	11.30-11.40
Session 1: Keynote Address **Professor Breandán Kennedy** School of Biomolecular & Biomedical Sciences, University College Dublin	**Chair, Prof Catherine Godson** *Unbiased drug discovery in zebrafish identifies therapeutic targets for human cancer and blindness*	11.40-12.10
Short Oral Communications	**12.10-13.10**	
Maura Naughton, UCC	*Decarboxylation of Ang-(1-7) to Ala1-Ang-(1-7) leads to major changes in pharmacodynamics*	12.10-12.22
Peter Crowley, UCD	*Comparison of general anaesthetic regimens on pulmonary and liver metastasis post-tumour resection surgery in a murine model of breast cancer*.	12.22-12.34
Dr Catríona Dowling, RCSI	*Discovery of a novel histone deacetylase 6 inhibitor that kills drug-resistant breast cancer*	12.34-12.46
Elaine Enright, UCC	*The influence of gut microbiota-mediated bile acid metabolism on the cellular response to therapeutics at the intestinal barrier*	12.46-12.58
Dr Aisling Williams, UCD	*Examining the Therapeutic Potential of JAK/STAT Signalling Manipulation in Human Cardiomyocytes*	12.58-13.10
Lunch Poster Viewing	Lunch and poster viewing/judging	13.10-14.15

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Contributor/Event	Title	Time
Session 2: Keynote Address **Dr Stephen Keely** **Associate Director, RCSI Molecular Medicine, Royal College of Surgeons in Ireland**	**Chair, Assoc. Prof John Crean** *Targeting bile Acids for Treatment of Intestinal Disease: From Ancient Miracle to Modern Medicine*	14.15-14.45
Keynote Address **Professor Jamie Coleman, University of Birmingham, BPS & EACPT**	*Clinical Pharmacology, past, present and future*	14.45-15.15
Session 3: Short Oral Communications	**Chair, Dr Anne-Marie Liddy**	15.15-16.15
Dr Monica de Gaetano, UCD	*Biological evaluation of the diverse anti-inflammatory effects of newly synthesised imidazole-/oxazole-containing Lipoxin A4 mimetics*	15.15-15.27
Michelle Lowry, TCD	*Investigating the mechanisms of neratinib-resistance in HER2-overexpressing breast cancer*	15.27-15.39
Dr Conor Daly, UCD	*A Brain-Derived Neurotrophic Factor Mimetic Is Sufficient to Restore Cone Photoreceptor Visual Function in an Inherited Blindness Model*	15.39-15.51
Anja Tetzner, UCC	*The AT2 receptor agonist, C21, can also stimulate Mas and MrgD receptors*	15.51-16.03
Dr Catherine Byrne, RCSI	*Anticholinergic and sedative drug burden in Irish community-dwelling older people: a cross-sectional national pharmacy claims database study*	16.03-16.15
Keynote Address **Dr Eamonn Molloy, Consultant Rheumatologist, St Vincent's University Hospital/UCD**	*Giant Cell Arteritis - what's the target?*	16.15-16.45
Reception	Announcement of prize winners & reception, Kevin Barry Gallery, Charles Institute	17:00

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LIST OF POSTERS

Poster	Abstract Summary
1	Novel molecular components of hippocampal neuronal plasticity revealed through analysis of the memory-associated transcriptional cascade. Abdulmalek S, et al. UCD
2	Connectivity Mapping defined therapeutics to target high risk proliferative myeloma. Coyne MRE, et al. School of Medicine, UCD
3	Platelet-derived microparticles of obese individuals induce endothelial-to-mesenchymal transition and produce higher levels of thromboxane A_2: a possible mechanism in obesity-driven tumorigenesis. Grande R, et al. d'Annunzio University, Chieti, Italy
4	In vitro model for studying the non-autonomous degeneration of neurons containing hyperphosphorylated tau: Relevance to Alzheimer's disease. Jaisimha A, et al. UCC
5	Proton pump inhibitors: ubiquitous and overused? Kennedy C, et al. Beaumont Hospital and Mater Hospital, Dublin
6	De Novo Vitamin D supplement use post-diagnosis reduces breast cancer mortality. Madden J, et al. RCSI and TCD
7	Degradation of Ang-(1-7) in different mouse organs. Moore A, et al. UCC
8	Nefiracetam is a myelin repair agent in vitro and in animal models of demyelinating disease. Murphy K et al. UCD
9	Cardiovascular risk predictors, QRISK2 and statins. Nicol P, et al. QUB
10	Hippocampal memory mechanisms are engaged by addictive drugs prior to the emergence of dependence behaviour. O'Sullivan J, et al. UCD
11	Developing zebrafish CRISPR/Cas9 knockout models to investigate the role of cysteinyl leukotriene receptors in ocular biology. Slater K, et al. UCD
12	Identify novel therapies for vascular disease and regenerative medicine based on the landmark discovery of induced pluripotent stem cells. Sterritt R, et al. QUB
13	Identification of novel therapeutic targets for treatment of inherited retinal dystrophies in zebrafish models. Sundaramurthi H, et al. UCD
14	The risk of QTc prolongation in hospitalized patients on methadone maintenance therapy. Tan B, et al. TCD
15	Host and environmental factors influencing expression of bacterial-derived metabolic enzymes in faeces: Potential implications for microbiota-mediated drug metabolism. Walsh J, et al. UCC
16	Investigation into the underlying aetiology of a visual function defect in a zebrafish model of von Hippel-Lindau disease. Ward R, et al. UCD
17	Elucidating the role of anaesthetic/analgesic target receptors in breast cancer metastasis. Xue C, et al. UCD

Keynote Address: Professor Breandán Kennedy, School of Molecular & Biomedical Sciences, University College Dublin

Unbiased drug discovery in zebrafish identifies therapeutic targets for human cancer and blindness

Biography:

Breandán Kennedy graduated with a BSc in Pharmacology from UCD in 1993. He completed his PhD at the W.Alton Jones Cell Science Center, New York and post-doctoral training in the University of Notre Dame, Indiana and University of Washington, Seattle before returning to UCD in 2003 as a College Lecturer in Pharmacology.

He is currently Professor of Pharmacology in the UCD School of Biomolecular and Biomedical Science, and a Conway Fellow. He is the co-founder of Phision Therapeutics which won the UCD Start-Up Award in 2015.

His primary research interests are in the development of genetic and pharmacological treatments for human blindness. Using zebrafish as an in vivo system, his group have identified several families with inherited blindness and have used these to characterise disease progression and evaluate therapies. They have also developed in vivo assays enabling us to discover novel drugs with specific neuoprotectant, anti-angiogenic or toxic properties in the eye.

Maura Naughton, Department Pharmacology & Therapeutics, University College Cork

Decarboxylation of Ang-(1-7) to Ala1-Ang-(1-7) leads to major changes in pharmacodynamics

Naughton M[1], Tetzner A[1], Gebolys K[1], Sala E[2], Villacañas Ó[2], Walther T[1, 3]

[1] Department Pharmacology & Therapeutics, School of Medicine and School of Pharmacy, University College Cork, Ireland; [2]Intelligent Pharma, Barcelona, Spain; [3]Institute of Medical Biochemistry and Molecular Biology, University Medicine Greifswald, Greifswald, Germany.

Within the renin-angiotensin system, angiotensin (Ang)-(1-7) is cardiovascular protective, stimulates regeneration, and opposes the often detrimental effects of Ang II. We identified two receptors for the heptapeptide; the G-protein-coupled receptors Mas and MrgD.

Recently, a decarboxylated form of Ang-(1-7), Ala1-Ang-(1-7), has been described as having similar vasorelaxing effects as Ang-(1-7) but distinctively stimulating the MrgD receptor.
The aim of this study was to elucidate the consequences of the lack in the carboxyl group in amino acid one on intracellular signalling, to discover the receptor fingerprint for Ala1-Ang-(1-7), and to characterize the consequences for pharmacodynamics. Using cAMP as readout, we showed that Ala1-Ang-(1-7) elevated cAMP concentrations in primary endothelial and mesangial cells. However, the dose-response curves clearly discriminated from the curvesgenerated with Ang-(1-7), with much lower EC50 and bell-shape for Ala1-Ang-(1-7).

We provided pharmacological proof that both, Mas and MrgD, are functional receptors for Ala1-Ang-(1-7). Consequently, the heptapeptide failed to increase cAMP concentration in primary mesangial cells with genetic deficiency in both receptors. As for Ang-(1-7), the AT2 blocker PD123319 also blocked the Ala1-Ang-(1-7) effects on Mas and MrgD receptors and in primary cells. The very distinct dose-response curves for both heptapeptides could be explained by in silico modelling, energy calculations, and an involvement of Galpha i for higher concentrations of Ala1-Ang-(1-7).

Our results identify Ala1-Ang-(1-7) as a peptide with specific pharmacodynamic properties and build the basis for the design of more potent and efficient Ang-(1-7) analogues for therapeutic interventions in a rapidly growing number of diseases.

Peter Crowley School of Medicine, Conway Institute of Biomolecular and Biomedical Research, University College Dublin

Comparison of general anaesthetic regimens on pulmonary and liver metastasis post-tumour resection surgery in a murine model of breast cancer.

Crowley PD[1], Johnson MZ[1,2], Foley AG[3], Xue C[1], Connolly CD [1,2], Buggy J[1,2, ,4,5] Gallagher HC [1,4].

[1]School of Medicine, Conway Institute of Biomolecular and Biomedical Research, University College Dublin; [2]Dept. of Anaesthesia, Mater University Hospital, School of Medicine, University College Dublin; [3]BerandNeuropharmacology Ltd., Nova UCD, Belfield Innovation Park, Dublin 4; [4]UCD-Mater Clinical Research Centre, Nelson St, Dublin 7; [5]Outcomes Research, Cleveland Clinic, OH, USA.

Breast cancer accounts for 7% of female cancer deaths. While surgery is the mainstay of treatment, perioperative interventions, such as anaesthesia, may influence risk of metastasis during breast tumour resection. However, no conclusive, prospective clinical or pre-clinical studies have been completed. Here, in the 4T1 model of breast cancer, we investigated the effects of sevoflurane, ketamine and xenon anaesthesia, as well as IV lidocaine, on metastatic burden and serum cytokines post-tumour resection surgery.

4T1 tumour cells were injected into the mammary fat-pad of female BALB/c mice. After 7 days, the resultant tumour was excised under sevoflurane,ketamine/xylazine, sevoflurane/ketamine/xylazine, or xenon/ketamine/xylazine anaesthesia. Four additional groups were anaesthetised with either sevoflurane or ketamine/xylazine in combination with peri-operative IV lidocaine or NaCl. Fourteen days post-surgery, animals were culled, organs harvested and lung and liver specimens were examined for metastasis. Pro-inflammatory and pro-metastatic cytokines were profiled using a slide-based array
(RayBiotech) in post-mortem serum.

Inclusion of peri-operative lidocaine reduced lung metastatic colony count versus sevoflurane with NaCl (median (IQR)), 0(0-2)vs 22.5(0-481), p=0.02, n=21 and reduced the proportion of animals with pulmonary metastasis, (28.5%vs 52.5%, p=0.04, n=21). However, there was no significant change in metastasis between mice anaesthetised with sevoflurane, ketamine/xylazine, sevoflurane/ketamine/xylazine, or xenon/ketamine/xylazine general anaesthesia (10≥n≤12).Serum analysis demonstrated reduced pro-inflammatory and angiogenic cytokine expression in animals receiving lidocaine with sevoflurane and a slight reduction in G-CSF in ketamine-treated animals compared with sevoflurane.

In this 4T1 murine model of breast cancer, lidocaine decreased pulmonary metastasis, when combined with sevoflurane anaesthesia. However, choice of general anaestheticdid not influence post-surgical metastasis to the liver or lungs.

Dr Catríona Dowling Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland

Discovery of a novel histone deacetylase 6 inhibitor that kills drug-resistant breast cancer

Dowling C[1], Hemann M[2], Bradner J[3], Letai A[4], Ni Chonghaile T[1]

[1]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin; [2]Koch Institute for Integrative Cancer Research at MIT, Massachusetts Institute of Technology, Cambridge, Massachusetts; [3] Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston; [4]Division of Hematologic Malignancies and Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA.

Triple negative breast cancer (TNBC) lacks expression of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2). Importantly, there have been fewer advances in the treatment of TNBC and new therapies are required to treated relapsed TNBC. We previously found that chemoresistant cancers are far from the mitochondrial apoptotic threshold. To identify novel therapeutics for the treatment of chemoresistant breast cancer, we performed a high-throughput screen to identify small molecules that are cancer selective but can kill independent of the mitochondrial pathway of apoptosis. We screened a total of 30,000 compounds in duplicate across the two cell lines in which the pro-death proteins BAX/BAK were knocked down.

There was a hit rate of 0.3% in the screen and 85 compounds were retested in the validation cherry pick screen. From this screen 18 compounds were further validated with low-throughput assessment for mitochondrial independent killing and selectivity for cancer cells. To identify the mechanism of action of the lead compound we used a genetic approach to generate an RNAi signature for the compound and it clustered with the histone deacetylase inhibitors (HDAC). Using an in vitro HDAC inhibitor screen, we identified that the compound selectively inhibited HDAC6.

To understand the biological signalling of the compound, we measured global acetylation by a novel mass spectrometry approach and identified known HDAC6 substrates, such as a-tubulin, as well as novel substrates. In conclusion, we have identified a novel HDAC6 specific inhibitor that selectively kills cancer cells independent of mitochondrial apoptosis.

Elaine Enright, School of Pharmacy, University College Cork

The influence of gut microbiota-mediated bile acid metabolism on the cellular response to therapeutics at the intestinal barrier

Enright EF[1], Govindarajan K[2], Darrer R[2], MacSharry J [2, 4, 5], Griffin BT[1], Joyce SA[2, 3*] Cormac G.M. Gahan CGM [1, 2, 4*]

[1]School of Pharmacy, University College Cork, Cork, Ireland [2]APC Microbiome Institute, University College Cork, Cork, Ireland [3]School of Biochemistry and Cell Biology, University College Cork, Cork, Ireland [4]School of Microbiology, University College Cork, Cork, Ireland [5]School of Medicine, University College Cork, Cork, Ireland.

Once regarded obscure, the cohabitation of man and microbe has gained increasing recognition as a determinant of the health status of the host. To date, pharmacokinetic research at the host-microbe interface has been primarily directed towards effects on metabolism1. Microbial bile acid (BA) metabolism, deconjugation and dehydroxylation of the steroidal nucleus by the gut bacteria, may constitute a source of pharmacokinetic variability, and has been shown to impact bile acid solubilization capacity2. The purpose of this work was to elucidate other possible mechanisms by which BAs, traditionally regarded to be surfactants, could affect intestinal drug absorption. This work investigated if host and microbial BAs could differentially influence intestinal drug transporter expression and thereby drug uptake.

The impact of microbial BA metabolism on the expression of common influx and efflux transporters (including ABCB1, encoding P-gp) in Caco-2 cells was assessed. The ability of host (conjugated) and microbial (deconjugated/dehydroxylated) BAs to differentially affect drug uptake and activity was investigated using the P-gp substrates, cyclosporine A (CsA) and rhodamine 6G (Rho 6G). Cell viability was used as a preliminary marker of altered CsA uptake/activity. Potential mechanisms by which BAs could affect P-gp functioning was evaluated using ATPase and bidirectional transport assays.
Unconjugated BAs significantly augmented CsA toxicity and reduced Rho 6G efflux, compared to tauro-conjugates ($P < 0.05$). These effects could not be explained by changes to ABCB1 mRNA transcripts. BAs were determined to inhibit, rather than stimulate, basal P-gp ATPase suggesting a non-competitive interaction with the transporter.

Microbial BA metabolism was demonstrated to affect the uptake and activity of efflux transporter substrates. The physicochemical properties of unconjugated bile acids, including their capacity for passive membrane diffusion, is speculated to underpin their preferential attenuation of P-gp-mediated efflux.

1. Enright, E. F.; Gahan, C. G.; Joyce, S. A.; Griffin, B. T. The Impact of the Gut Microbiota on Drug Metabolism and Clinical Outcome. The Yale journal of biology and medicine 2016, 89, (3), 375-382.

2. Enright, E. F.; Joyce, S. A.; Gahan, C. G.; Griffin, B. T. Impact of Gut Microbiota-Mediated Bile Acid Metabolism on the Solubilization Capacity of Bile Salt Micelles and Drug Solubility. Molecular pharmaceutics 2017, 14, (4), 1251-1263.

Dr Aisling Williams, University College Dublin

Examining the Therapeutic Potential of JAK/STAT Signalling Manipulation in Human Cardiomyocytes

Williams A[1], Tobin-O'Brien C[1], O'Sullivan KE [2, 3,], MacDonagh L[1], Breen E[1], Buggy DJ[1], Hurley JP[2], Gallagher HC [1,3] .

[1] University College Dublin, Dublin, Ireland [2] Mater Private Hospital, Dublin, Ireland, [3] Mater Misericordiae University Hospital, Dublin, Ireland.

Recently, iPS-derived human cardiomyocytes have been validated as an authentic model for the prediction of in vivo functional cardiotoxicity. To date, there have been no previous studies with these cells to examine the effects of hypoxia, despite the high clinical burden associated with ischemic heart disease. Here, we used commercially available Cor.4U iPS-derived cardiomyocytes (Axiogenesis) to assess signaling pathways and functional parameters involved in the hypoxic response. Cardiomyocytes were grown until beating synchronously, followed by exposure to physiological normoxia (10% O2) or hypoxia (1% O2) for time-periods up to 16h. Direct video microscopy combined with image analysis software (Cellogy, Pulse) was used to determine effects on beating.

Functional toxicity was evident within two hours of hypoxic exposure, evident by a decrease in beating rate of 10% which further decreased to 40% of normal after 16h. Induction of hypoxia inducible factor alpha (HIF-α) confirmed that cells were under hypoxic stress. In normoxic conditions, functional toxicity was not observed with the beating rate remaining at 85% after 16 h. Interestingly, exposure to 100ng/ul insulin-like growth factor 1 (IGF-1), which is known to activate JAK/STAT and other signalling pathways protected the cardiomyocyte against the effects of hypoxia on beating inducing a sustained 40% increase under hypoxic and normoxic conditions. We also saw protective affects from PI3K inhibitor, GDC-0941 (33nM). Other JAK/STAT activators and inhibitors effected the the beating strength of the cardiomyocytes.

These results will help generate new therapeutic strategies aimed at minimizing the damage to the cardiomyocyte induced during ischemia/reperfusion injury.

Keynote Address: Dr Stephen Keely, Associate Director, Molecular Medicine, RCSI, Royal College of Surgeons in Ireland.

Targeting bile acids for treatment of intestinal disease: From Ancient Miracle to Modern Medicine

Biography:

Dr. Keely carried out his PhD studies in the Dept. of Pharmacology, University College Dublin, before moving to the Dept. of Medicine at the University of California, San Diego, for his postdoctoral training. He returned to Ireland in 2005 to take up the position of Associate Director of the Dept. of Molecular Medicine at the Royal College of Surgeons in Ireland. Dr. Keely's research is focussed on developing our understanding of how bile acids regulate intestinal transport and barrier function and how they contribute to the pathophysiology of intestinal diseases, such as inflammatory bowel disease and colorectal cancer. Moreover, he is interested in how bile acids interact with our resident intestinal microbiota, to ultimately influence epithelial cell survival, transport and barrier function, and immune interactions within the mucosa. Ultimately, his work aims to identify new approaches to target bile acids and their receptors in the treatment of chronic intestinal disease.

Keynote Address: Professor Jamie Coleman, University of Birmingham, British Pharmacological Society, European Association for Clinical Pharmacology and Therapeutics.

Clinical Pharmacology, past, present and future.

Biography:
Jamie Coleman is Professor of Clinical Pharmacology and Medical Education at the University of Birmingham, UK.

A current British Pharmacological Society Clinical Section Fellow, Jamie enjoys a wide range of educational and research responsibilities in the UK. Jamie is Deputy Programme Director and Therapeutics Lead Teacher for the third largest undergraduate medical degree in the UK, and also chairs the Assessment Board of the highly successful Prescribing Safely Assessment in the UK and Ireland.

As a recognised National and International expert in electronic prescribing, he also inputs to national strategy in digital medicines management and is Co-Principal Investigator on a large research programme investigating ePrescribing effects on medication safety in England. Other responsibilities include chairing the UK National Speciality Advisory Committee for doctors training in CPT and providing input to the pharmacovigilance expert advisory group and licensing committee meetings at the MHRA. He is also Chairperson Elect of the European Association of Clinical Pharmacology and Therapeutics Executive Committee.

Dr Monica de Gaetano, School of Medicine and Medical Sciences, Diabetes Complication Research Centre, UCD Conway Institute, University College Dublin

Biological evaluation of the diverse anti-inflammatory effects of newly synthesised imidazole-/oxazole-containing Lipoxin A4 mimetics

de Gaetano M[1], Butler EM[2], Gahan K[2], Zanetti A[2], Marai M[1], Maingot C[3], McLoughlin A[3], Hams E[4], Leroy X[5], Fallon P[4], Loscher CE[3], Guiry PJ[2] Godson C[1]

[1]School of Medicine and Medical Sciences, Diabetes Complication Research Centre, UCD Conway Institute, University College Dublin; [2]Centre for Synthesis and Chemical Biology, School of Chemistry, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland; [3]School of Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland; [4]Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland; [5]Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, 4123 Allschwil, Switzerland.

Background: Lipoxins [LXs] are endogenously generated eicosanoids with potent bioactions consistent with attenuation of inflammation. We have previously demonstrated that LXs can promote the resolution of inflammation. The costly synthesis and metabolic instability of LXs may limit their therapeutic potential. Here we report synthesis and characterization of novel imidazole-/oxazole-containing LX-mimetics.

Methods: The key steps of asymmetric synthesis of putative LX-mimetics include a Suzuki reaction and an asymmetric ketone reduction. The effect of the novel compounds on inflammatory responses was assessed (a) using a human monocyte cell line stably expressing an NFkB reporter gene, (b) by investigating downstream cytokine secretion.
Responses were assayed relative to endogenous LXs and dexamethasone (proto-typic anti-inflammatory molecule).

The potential interaction of the imidazoles/oxazoles with the molecular target of LXs (i.e. GPCR ALX/FPR2) was investigated using a cell system where ALX/FPR2 is coupled to Gq and receptor interaction determined by mobilization of intracellular calcium.
In vivo anti-inflammatory effects were assessed using a murine zymosan-induced peritonitis model.

Results: Overall, structure-activity relationship studies demonstrated that the (R)-epimer of 6C-dimethyl-imidazole was the most potent and efficient anti-inflammatory agent, among the ten compounds tested. This molecule significantly attenuated LPS-induced NFkB activity [ECmax=1pM, 44% reduction, $p<0.01$], reduced the release of several pro-inflammatory cytokines and inhibited peritonitis-associated neutrophil infiltration in vivo. The underlying mechanism for those actions appeared to be through FPR2 activation. No cytotoxicity of the mimetics was detected. Conclusions These data support the therapeutic potential of imidazole-containing LX-mimetics in the context of novel inflammatory regulators.

(Work supported by Science Foundation Ireland)

Michelle Lowry, School of Pharmacy and Pharmaceutical Sciences, Trinity Biomedical Sciences Institute, Trinity College Dublin

Investigating the mechanisms of neratinib-resistance in HER2-overexpressing breast cancer

Lowry MC [1], Breslin S [1], Toomey S[2], Hennessy BT[2], O'Driscoll L[1]

[1] School of Pharmacy and Pharmaceutical Sciences, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2; [2]Medical Oncology Group, Department of Molecular Medicine, Royal College of Surgeons in Ireland, Dublin.

HER2+ breast cancer accounts for 20-25% of all breast cancers. In July 2017, the FDA approved neratinib for the extended adjuvant treatment of adult patients with early-stage HER2+ breast cancer. Although neratinib is proving efficacious, neratinib-resistance (NR) is an evolving issue and the mechanisms need to be deciphered.

NR variants of three HER2+ cell lines (EFM19.2A, HCC1954 and SKBR3) were developed by exposing cells to increasing concentrations of neratinib over a 6-month period. Acid phosphatase assays were used to determine neratinib IC50 values. Ultracentrifugation was used to purify extracellular vesicles (EVs) released from each cell variant. EVs were characterised by immunoblotting, TEM and nanosight tracking analysis. Cellular DNA and EV DNA content were investigated using Sequenom MALDI-TOF MS. Cell and EV content of 181 proteins were sequenced by Olink and further validated by immunoblotting.

NR variants of the 3 cell lines were successfully developed, as EFM19.2A-NR, HCC1954-NR and SKBR3-NR. The neratinib IC50 values were 6.5-fold, 6.8-fold and 7.4-fold that of their respective parent cell lines. EVs were isolated and characterised. DNA Sequenom led to the discovery of 3 SNPs in cell line pairs (HCC1954 and EFM19.2A). Three proteins (CA9, CSF-1 and TLR3) showed substantial increased quantities in NR variants and their respective EVs, compared to their drug-sensitive counterparts. Immunoblots were completed to validate these findings.

Further studies are warranted to validate these findings, to investigate the functional relevance of CA9, CSF-1 and TLR3 in NR and, subsequently, progress our findings to analysis of specimens from cohorts of breast cancer patients.

Dr Conor Daly, School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin

A Brain-Derived Neurotrophic Factor Mimetic Is Sufficient to Restore Cone Photoreceptor Visual Function in an Inherited Blindness Model

Daly C[1] , Shine L[1] , Heffernan T[1] , Deeti S[1] , Reynolds AL[1] , O'Connor JJ[1] , Dillon ET [1,2], Duffy DJ[3], Kolch W [1,3,4], Cagney G[1] Kennedy BN[1].

[1]UCD School of Biomolecular & Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland; [2]Food for Health Ireland, Science Centre South, University College Dublin, Belfield, Dublin 4, Ireland; [3] Systems Biology Ireland, Science Link Building, University College Dublin, Belfield, Dublin 4, Ireland; [4] School of Medicine, University College Dublin, Belfield, Dublin 4, Ireland.

Controversially, histone deacetylase inhibitors (HDACi) are in clinical trial for the treatment of inherited retinal degeneration. Utilizing the zebrafish dyeucd6 model, we determined if treatment with HDACi can rescue cone photoreceptor-mediated visual function. dye exhibit defective visual behaviour and retinal morphology including ciliary marginal zone (CMZ) cell death and decreased photoreceptor outer segment (OS) length, as well as gross morphological defects including hypopigmentation and pericardial oedema. HDACi treatment of dye results in significantly improved optokinetic (OKR) (~43 fold, $p<0.001$) and visualmotor (VMR) (~3 fold, $p< 0.05$) responses.

HDACi treatment rescued gross morphological defects and reduced CMZ cell death by 80%. Proteomic analysis of dye eye extracts suggested BDNF-TrkB and Akt signaling as mediators of HDACi rescue in our dataset. Co-treatment with the TrkB antagonist ANA-12 blocked HDACi rescue of visual function and associated Akt phosphorylation. Notably, sole treatment with a BDNF mimetic, 7,8-dihydroxyflavone hydrate, significantly rescued dye visual function (~58 fold increase in OKR, $p<0.001$, ~3 fold increase in VMR, $p<0.05$).

In summary, HDACi and a BDNF mimetic are sufficient to rescue retinal cell death and visual function in a vertebrate model of inherited blindness.

Anja Tetzner, Department Pharmacology & Therapeutics, University College Cork

The AT2 receptor agonist, C21, can also stimulate Mas and MrgD receptors

Tetzner A[1*], Naughton M[1*], Gebolys K[1], Sala E[2], Villacañas Ó[2], Walther T[1, 3]

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*Equally contributing first authors

It is well accepted that Compound-21 (C21) is a highly selective non-peptide AT2 receptor agonist.

C21 as well as angiotensin (Ang)-(1-7) have cardiovascular protective effects and are opponents of the often detrimental AngII within the renin-angiotensin system. Since the chemical structure of
C21 is similar to the Mas receptor specific non-peptidic agonist AVE0991, and our recent finding that the AT2 receptor blocker, PD123.319, can also block the effects of Ang-(1-7) on Mas and MrgD, we tested whether C21 is also not AT2-specific but can stimulate the two Ang-(1-7) receptors.

Using cAMP as readout in receptor-transfected HEK293-cells, we generated pharmacological proof that Mas (EC50=1.995x10-12M) and MrgD (EC50=2.958x10-9M) are functional receptors for C21, whereby the three receptor blockers, A779, D-Pro7-Ang-(1-7), and PD123.319 showed receptor- specific effects towards C21 signalling. Furthermore, C21 elevated the cAMP concentration in primary cells such as mesangial cells (EC50=1.12x10-6M). However, significant increase in cAMP levels, but not in PKA activity, was still detectable in mesangial cells isolated from AT2-deficient mice, but completely blunted in Mas/MrgD-double knockouts. Finally, in silico modelling was performed to illustrate the structural similarities and differences between C21, AVE0991, and Ang-(1-7).

Our results identify C21 as not being a specific AT2 receptor agonist, but also interacting with the two Ang-(1-7) receptors, Mas and MrgD. Therefore, the partial overlap in beneficial effects of Ang-(1-7) and C21 might be based on the stimulation of the same receptors under specific pathophysiological circumstances. This also enforces the revisit of such publications which concluded on AT2 function by only using C21.

Dr Catherine Byrne, Division of Population Health Sciences, Royal College of Surgeons in Ireland

Anticholinergic and sedative drug burden in Irish community-dwelling older people: a cross-sectional national pharmacy claims database study

Byrne C[1], Walsh C[1], Cahir C[1], Ryan C[2], Williams DJ[3], Bennett K[1].

[1]Division of Population Health Sciences, Royal College of Surgeons in Ireland, Dublin, Ireland; [2]School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Ireland; [3]Department of Geriatric and Stroke Medicine, Royal College of Surgeons in Ireland and Beaumont Hospital, Dublin, Ireland.

Aim: To examine the prevalence of exposure to anticholinergic and sedative medications in Irish community-dwelling older people in 2016 using the Drug Burden Index (DBI) tool, and the association of patient factors with exposure.

Methods: A list of medications with clinically relevant anticholinergic and/or sedative effects was developed from drug monographs (DBI medications). Prevalence rates were calculated for those aged ≥65 years, eligible for the General Medical Services (GMS) scheme and in receipt of at least one pharmacy claim in 2016. Multivariate logistic regression was used to examine the association of patient age and sex with exposure to DBI medications. Odds ratios (OR) and 95% confidence intervals (CI) are presented.

Results: Overall, 285,381 of the 438,283 (65.1%) GMS eligible older population received at least one pharmacy claim for a DBI medication in 2016. Prevalence of exposure to DBI medications was significantly higher in women compared to men (70.7% vs. 57.8% respectively, OR 1.74, 95% CI 1.72-1.76). Those aged ≥80 years had a significantly higher prevalence of exposure than those aged <80 years (70.8% vs. 62.7% respectively, OR 1.42, 95% CI 1.39-1.44). The most frequently dispensed DBI medications were codeine/paracetamol combinations (18.5%), zopiclone (9.0%), zolpidem (8.0%), pregabalin (7.5%) and alprazolam (7.3%).

Conclusion: The prevalence of exposure to DBI medications in Irish community-dwelling older patients is high, particularly in women and the very old. Future research should focus on examining the impact of DBI on clinical outcomes in older people and developing interventions to reduce inappropriate prescribing of anticholinergic and sedative medications.

Keynote Address: Dr Eamonn Molloy, Consultant Rheumatologist, St Vincent's University Hospital, University College Dublin.

Giant Cell Arteritis – what's the target?

Biography:
Eamonn Molloy graduated from University College Dublin (1997) and completed rheumatology and internal medicine training in Ireland. He obtained an MD at RCSI (2006), which focussed on calcium crystal-induced inflammation.

From 2005, he underwent subspecialty fellowship training in vasculitis at the Cleveland Clinic, completed an MS (Clinical Research) at Case Western Reserve University and then joined the staff at the Vasculitis Center and FJ Fasenmeyer Center for Clinical Immunology at the Cleveland Clinic. In 2010, he was appointed as a consultant rheumatologist at St Vincent's University Hospital and is a UCD Senior Clinical Lecturer.

He is author of over 60 publications largely pertaining to vasculitis, complications of biologic therapy and crystal-induced arthritis. Currently, his primary research focus is giant cell arteritis.

POSTER PRESENTATIONS

Sarah Abdulmalek, Neurotherapeutics Research Group, School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin

Novel molecular components of hippocampal neuronal plasticity revealed through analysis of the memory-associated transcriptional cascade.

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A learning event initiates a cascade of molecular signals, which culminates in alterations in gene activity. These, in turn, mediate synaptic remodelling, in particular, within the hippocampus, a structure vital to memory formation. Previous studies suggest that early phases (0-6h) of memory consolidation involve synaptic preparation by internalization of adhesion molecules, followed later by synaptic restructuring involving synaptic growth (6-12h) followed by selection (12h+). The molecular underpinnings of these morphological events are still poorly understood.

Previously, we employed DNA microarrays to study the mRNA expression profiles of the rat dentate gyrus at increasing times following either water maze spatial learning or avoidance conditioning. Here, we report that 609 and 700 genes were transcriptionally regulated by ≥30% and ≥20% across the 24h post-training period following spatial learning and avoidance conditioning, respectively. When these gene lists were compared, a cohort of 135 transcripts was identified, whose expression levels are regulated following both learning tasks.

Collectively, these genes could be considered to be a core transcriptional program for memory consolidation that is deployed independent of the nature of the learning task. We show that two members of the cluster, midkine and klotho, can increase neurite outgrowth and complexity in primary hippocampal cell culture and that klotho is upregulated by the memory-associated neurotransmitter glutamate.

Finally, we show that midkine can enhance learning and memory in the water maze. These studies identify a memory-associated gene cluster, members of which can drive memory consolidation in vivo.

Dr Mark Coyne, Clinical Pharmacology and Therapeutics, University College Dublin; Department of Haematology, Mater Misericordiae University Hospital, Eccles Street, Dublin 7.

Connectivity Mapping defined therapeutics to target high risk proliferative myeloma

Coyne MRE [1, 2]

[1]Clinical Pharmacology and Therapeutics, University College Dublin, Belfield, Dublin 4; [2]Department of Haematology, Mater Misericordiae University Hospital, Eccles Street, Dublin 7.

i: In myeloma, a relative increase in proliferation confers the worst overall survival. Drugs targeting proliferation are not part of common treatment algorithms. This study utilises the in-silico method of Connectivity Mapping (Cmap) to source potential therapeutics for this poor risk cohort.

ii: GSE24080 a publically available dataset was utilised. GSE24080 consists of CD138 purified RNA from 560 patients undergoing total therapy 2 and 3 at University of Arkansas Medical Sciences. The dataset was first transformed using a RMA algorithm, then filtered, based on the individual patients TC class. TC classification was calculated using the MAS5 dataset. Within each TC class, patients were further catergorised based on their gene expression proliferation index as either high or low. Differentially expressed genes between those with a high proliferation index and low proliferation index were calculated. The intersection of the set of genes differentially expresssed with a p-value < 0.05 and absolute 2-fold change were obtained. This revealed 90 genes associated with the proliferation phenotype. These 90 genes were inputting into the Cmap hosted at the Broad Institute.

iii: mTOR inhibitors, HDAC inhibitors and calcium channel blockers are predicted to target this high risk cohort.

iv: Cmap is a useful hypothesis generating tool. mTOR inhibitors, HDAC inhibitors and calcium channel blockers require further confirmatory experiments to establish if they successful target this high-risk cohort in myeloma.

Rosalia Grande, Department of Neurosciences, Imaging and Clinical Science, Section of Cardiovascular and Pharmacological Sciences and Center of Aging Sciences & Translational Medicine, G. d'Annunzio University, Chieti, Italy.

Platelet-derived microparticles of obese individuals induce endothelial-to-mesenchymal transition and produce higher levels of thromboxane A2: a possible mechanism in obesity-driven tumorigenesis

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The biological mechanisms underlying the link between excess body weight and increased risk of developing cancer (Kyrgiou et al. 2017) have not been elucidated yet. We hypothesized that platelet-derived MPs (PMPs) play a role in cancer development and progression by inducing endothelial-mesenchymal transitions (EndMT).

We aimed to characterize PMPs isolated from obese versus healthy subjects for their capacity to generate thromboxane(TX)A2, potent stimulus for platelets activation, and for the expression of CD61, platelet receptor subunit of glycoproteinIIb/IIIa. Furthermore, we compared the capacity of obese or healthy subjects PMPs to induce EndMT in vitro. MPs (250 MPs/µl), isolated from thrombin-stimulated platelets (Vasina et al. 2013), were cultured alone or with Human Microvascular Endothelial Cells (HMVEC, 2x105) for 24h. Healthy volunteers PMPs cultured alone released TXB2 in a time-dependent fashion. Obese individuals PMPs cultured for 24h released TXB2 levels higher respect healthy PMPs.

Only in obese individuals, the levels of TXB2 released from PMPs positively correlated with the expression of CD61 on PMP surface. Obese individuals PMPs released a higher amount of TXB2 even co-cultured with HMVEC for 24h, and EndMT occurred in the co-culture versus untreated cells, as shown by the down-regulation of VE-cadherin and up-regulation of α-smooth muscle actin.

Our findings show that obese individuals PMPs produce higher levels of TXA2 and have a higher capacity to induce EndMT in HMVEC than healthy PMPs. Our results open the way to perform further studies to verify whether antiplatelet drugs play an antitumorigenic effect in obesity-driven cancer by preventing PMPs-dependent EndMT induction.

Anirudh Jaisimha Department Pharmacology & Therapeutics, University College Cork

In vitro model for studying the non-autonomous degeneration of neurons containing hyperphosphorylated tau: Relevance to Alzheimer's disease

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The hyperphosphorylation of tau precedes its conversion into paired helical filaments (PHFs) and neurofibrillary tangles (NFTs) in the Alzheimer's disease (AD) brain. PHF-1 is an antibody that specifically detects the Ser396/Ser404 phospho-epitope of tau (p-tau), which is known to correlate with Braak stage severity and neurodegeneration. In this study, we found that embryonic (E18) rat primary cortical neurons grown in Neurobasal® medium (NBM + 2% B27) contained high amounts of PHF-1-positive tau throughout a two-week in vitro study (DIV1 - 14). Despite having a high PHF-1: non-phosphorylated tau (Tau 1) ratio, cultured neurons established and maintained an elaborate network of neurites up to DIV14.

However, the presence of neuronal F-actin inclusions from DIV3 provided additional evidence of stress induced by in vitro culture conditions. Alterations in media glucose concentration above the standard 25mM contained in the media (50mM and 100mM) or below (4mM), did not affect the PHF-1: Tau 1 ratio, indicating the in vitro stress wasglucose-independent. To determine the impact of astrocytes and microglia on PHF-1: Tau 1ratio and neuronal integrity, mixed neuron-glial cultures were grown in NBM + 2% B27 + 10% FBS. The heterogeneous presence of GFAP-positive astrocytes and Iba1-positive microglia did not affect the PHF-1: Tau 1 ratio at DIV3, 7, 10 and 14. However, evidence of progressive neurodegeneration was observed in mixed neuron-glial cultures from DIV10, as seen with synapse loss (synaptophysin), degeneration of neural networks (βIII-tubulin) and an increase in fractalkine expression preceding a reduction in neuronal number.

This study provides evidence that cultured neurons grown in Neurobasal medium undergo in vitro stress, which leads to the hyper phosphorylation of tau and formation of F-actin inclusions, two hallmarks of AD neuropathology. While these findings highlight the confounding variable of in vitro culture conditions in neurobiology research, the in vitro model we have developed can be useful for studying factors that initiate and modulate non-autonomous neurodegeneration in AD.

Dr Cormac Kennedy, Department of Geriatrics and Stroke Medicine, Beaumont Hospital, Dublin 9, and Acute Medical Unit, Mater Hospital, Dublin 7

Proton pump inhibitors: ubiquitous and overused?

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Introduction: Research suggests that 30-70% of proton pump inhibitor (PPI) prescriptions are inappropriate. Recent pharmacoepidemiological evidence has associated an increased risk of chronic kidney disease and dementia with chronic PPI therapy. This study aimed to improve patient care and cost effective medication use by reducing inappropriate PPI prescriptions.

Methods: At two hospital sites, the study recorded whether the patient was on a PPI, its name, dose, regimen, indication and medications at risk of causing gastrointestinal symptoms. Indications were compared to those in the PPI's summary of product characteristics. The results and recommendations were presented to prescribers. A PPI prescribing guideline was developed and published.

The study was approved by the hospital Research Ethics Committee. Results In total, 885 patients were included in the cohort. Prior to prescriber education, 60% of patients were prescribed a PPI, 31% did not have an appropriate indication. Following prescriber education, 14% less patients were prescribed a PPI ($p<0.05$) compared to the number on a PPI prior to the intervention. 13% more patients had an identifiable indication ($p<0.05$).

Conclusion: This study addressed the substantial issue of inappropriate PPI prescribing. The prescribers and pharmacists involved in the study as well of those at the educational sessions were encouraged to adopt guideline driven prescribing practices. A successful intervention was instituted to improve patient care while emphasising appropriate prescribing practices. References REPOSI Investigators (2011). Eur J Intern Med 22:205-210.

Dr Jamie Madden, Population Health Sciences Division, Royal College of Surgeons in Ireland

De Novo Vitamin D supplement use post-diagnosis reduces breast cancer mortality

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Epidemiological evidence of any protective effect of vitamin D on breast cancer progression is only emerging. This study investigates the association between vitamin D initiated after a breast cancer diagnosis and associated mortality. Women aged 50-80 years with a record of invasive breast cancer were identified on the National Cancer Registry Ireland database (n=5417). Initiation of de novo vitamin D post-diagnosis was identified from linked national prescription data (n=2581, 49%).
Multivariate Cox proportional hazards models were used to estimate adjusted HRs (95% CIs) for breast cancer-specific mortality.

There was a 20% reduction in breast cancer-specific mortality in de novo vitamin D users compared to non-users (HR, 0.80; 95% CI, 0.64-0.99 p=0.048) and the reduction was greater at 49% (HR, 0.51; 95% CI, 0.34-0.74, p<0.001), if vitamin D was initiated soon after the breast cancer diagnosis (within 6 months). Similarly, there was a reduction, after stratification by recommended daily intake (1-400 IU/day HR, 0.52; 95% CI, 0.43-0.63, p<0.001; 400 + IU/day HR, 0.38; 95% CI, 0.28-0.52, p<0.001) and in analyses stratified by duration of exposure (1-12months HR, 0.73; 95% CI, 0.60-0.91, p<0.003; >12months HR, 0.33; 95% CI, 0.26-0.41, p<0.001).

In this large national breast cancer cohort, de novo vitamin D use post-diagnosis was found to be associated with a reduction in breast cancer-specific mortality. Vitamin D, therefore, has the potential as a non-toxic and inexpensive agent to improve survival in breast cancer patients. Findings support the need for RCTs exploring the effect of vitamin D supplementation on breast cancer survival.

Andrew Moore, Department of Pharmacology & Therapeutics, University College Cork

Degradation of Ang-(1-7) in Different Mouse Organs

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Angiotensin (Ang)-(1-7) has cardioprotective effects that serve to counter-regulate adverse effects of Ang II in the cardiovascular system. Ang-(1-7) inhibits the proliferative, cell growth promoting, and pressor effects of Ang II. Furthermore, it shows anti-fibrotic and anti-hypertrophic properties in preclinical models of myocardial infarction.

It is well accepted that Ang-(1-7) is primarily degraded to Ang-(1-5) by ACE in the cardiovascular system. However, we have preliminary results showing variations exist in Ang-(1-7) degradation pathways in various mouse organs. It is the aim of this study to quantify the metabolites in individual organs and to generate an organ-specific fingerprint of Ang-(1-7) truncation.

Our results show that after 10 minutes, Ang-(1-7) is degraded fastest in lung (1% of peptide remaining) and slowest in brain (86% of peptide remaining). Unsurprisingly, Ang-(1-5) was a major degradation product for organs with substantial ACE2 expression, such as lung and kidney. However, we identified Ang-(1-4) as another major degradation product in lung and kidney. In contrast, the major degradation product of Ang-(1-7) in the brain, ventricle, testis, and liver was Ang-(2-7). Ang-(1-7) was not at all converted in atrium membranes.

Ang-(1-7) degradation varies greatly in both speed and metabolites generated between mouse organs. The necessity to update the relatively old canonical Ang-(1-7) degradation pathway is paramount for drug design and efficacy. By identifying and subsequently inhibiting the peptidases ultimately responsible for the metabolism/catabolism of Ang-(1-7), its circulating concentration can be increased, thus improving the benefits of the heptapeptide's cardiovascular protective effects.

Dr Keith Murphy, Neurotherapeutics Research Group, UCD School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin.

Nefiracetam is a myelin repair agent in vitro and in animal models of demyelinating disease

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Multiple sclerosis (MS) is an autoimmune disease and the current therapies, all immune suppressants, only slow progression. Myelin repair offers an alternative target for intervention. The mechanism governing repair, migration and maturation of oligodendrocytes during recovery is still relatively poorly understood, making this a difficult target to exploit for drug development despite the tremendous potential of this approach.

Here, we report our data on nefiracetam, a compound never previously associated with myelin biology, which suggests that this compound can accelerate the spontaneous rate of myelin repair, both in vitro and in vivo. We show that nefiracetam can mediate myelin repair in the lysophosphatidylcholine (LPC)-induced myelin toxicity in vitro model in organotypic hippocampal slice culture across a range of concentrations.

In this in vitro model, nefiracetam clearly augments repair of myelin following damage rather than protection against such lesions. Nefiracetam can mediate myelin repair in vivo through a non-inflammatory mechanism in the cuprizone diet model of demyelination. In this study, treatment with nefiracetam mediated a significantly improved recovery of myelin in the corpus callosal pathway.

We have also confirmed that, in the classic MS in vivo model of EAE, if inflammation is controlled by the steroid anti-inflammatory dexamethasone, then nefiracetam can alleviate symptoms of motor impairment. Additionally, we show that nefiracetam can promote oligodendrocyte maturation in purified oligodendrocyte precursor cell culture. These data strongly support nefiracetam as a potential novel therapy that could significantly improve treatment of MS.

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Patrick Nicol, School of Medicine, Dentistry and Biomedical Sciences, Centre for Public Health, Institute for Health Sciences, Queen's University Belfast, Northern Ireland.

Cardiovascular Risk Predictors, QRISK2 and Statins

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Background: Cardiovascular risk predictors are used to estimate a patient's risk of cardiovascular disease in the next 10 years. Currently NICE advocates the QRISK2 calculator. The Framingham cardiovascular risk predictor has been outlined here as a possible alternative. Risk predictors have great implications for the treatment of patients with statin guidelines based on cardiovascular risk. This projects aim was to validate the use of QRISK2 as opposed to Framingham in a Northern Ireland population.

Design: This project was a cross sectional analysis of the QRISK2 model and the Framingham equation. Radioisotope scans of the heart were used to diagnose cardiovascular disease and compare risk scores.
Participants: 118 individuals between the ages of 43 and 84 with no previous history of cardiovascular disease recruited from a nuclear cardiology clinic in Belfast.

Results: QRISK2 predicted higher scores for both sexes in comparison with Framingham. The mean risk calculated by QRISK2 was 16.4% for women and 26.7% for men compared to 10.5% and 22.9% using Framingham. Left ventricular ejection fraction was inversely related with the risk scores calculated and showed Framingham to be better correlated with LVEF than QRISK2. QRISK2 displayed a higher sensitivity by identifying 47.8% of the participants with cardiovascular disease as 'high risk' (³20% risk).

Conclusion: This project showed that radioisotope scans of the heart are ineffective when evaluating cardiovascular risk predictors. Although Framingham was better correlated with left ventricular ejection fraction, QRISK2 was shown to be a better model for Northern Ireland due to its higher sensitivity.

Josiah O'Sullivan, Neurotherapeutics Research Group, UCD Conway Institute, School of Biomolecular and Biomedical Science, College of Science, University College Dublin

Hippocampal memory mechanisms are engaged by addictive drugs prior to the emergence of dependence behaviour

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Early mechanisms driven by drugs of abuse provide the foundation for initial continuation of drug intake, relate to memory of drug reward and underpin the major cause of relapse in reforming addicts – drug craving. Here we demonstrate memory-associated hippocampal neuroplastic activation and synaptic reorganisation in response to early treatments with addictive drugs.

Following daily drug exposure and the development of dependence, the neuroplastic response is lost. This pattern of hippocampal activity mirrors precisely the molecular events associated with learning a classical explicit memory task, the water maze, and the appearance of recall-based behaviour in that paradigm. Following the emergence of drug seeking behaviour and molecular adaptation, novel, non-drug-related hippocampal-dependent learning events are no longer processed effectively for long-term storage in the absence of the addictive drug suggesting dependence on the latter for normal memory function.

These studies demonstrate that drugs of abuse can initially activate memory encoding systems in the hippocampus while chronic exposure to such agents drives a molecular adaptation creating a situation where normal memory function is dependent on drug presence.

Kayleigh Slater, School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Ireland.

Developing zebrafish CRISPR/Cas9 knockout models to investigate the role of cysteinyl leukotriene receptors in ocular biology

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Ocular neovascularization and chronic inflammation are pathological hallmarks of debilitating ocular diseases including diabetic retinopathy, age related macular degeneration and glaucoma. There remains an unmet clinical need for effective anti-angiogenic and anti-inflammatory drugs which actively target these processes. Cysteinyl leukotriene receptors (e.g. CYSLT1 & CYSLT2) are known regulators of inflammation, angiogenesis and vascular permeability, making them attractive therapeutic targets for ocular disease. Understanding their function is of paramount importance for the development of new drug strategies or combination drug regimens for ocular disease.

Here, we are using genome-editing CRISPR/Cas9 technology to develop knockouts of cysteinyl leukotriene receptors in zebrafish to better understand their requirement for vision and their role in ocular disease.

CRISPR constructs for a deletion were designed, cloned into pDR274 expression vector and transformed into competent cells. Subsequently, they and Cas9 encoded within pT3TS-nCas9n expression vector were in vitro transcribed. The resulting mRNAs were co-injected into one cell-stage zebrafish embryos. Gene knockout was analysed by polymerase chain reaction (PCR) and DNA sequencing of F0 injected adult zebrafish.

Genotyping PCR and DNA sequencing results are consistent with a deletion event of approximately 600 and 780 bp in cysltr1 and cysltr3 respectively. Phenotypic characterisation of homozygous mutants will reveal requirement of these receptors for developmental angiogenesis in the eye, retinal pattering and visual behaviour.

Ryan Sterritt, School of Medicine, Dentistry and Biomedical Sciences, Centre for Experimental Medicine, Institute for Health Sciences, Queen's University Belfast, Northern Ireland.

Identify novel therapies for vascular disease and regenerative medicine based on the landmark discovery of induced pluripotent stem cells

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Cardiovascular disease (CVD) is the leading cause of death globally, in 2013 it was responsible for 30% of all deaths worldwide. It has been shown that endothelial dysfunction is a major pathological mechanism that precedes CVD, yet these damaged endothelial cells (ECs) display poor regenerative capabilities.

Current therapy is inadequate or unsuitable for a significant number of patients, the ability to replace damaged ECs could serve as a valuable therapeutic option for the future. Recent capability to generate induced pluripotent stem cells (iPSCs) and differentiate these towards ECs holds great promise in the area of regenerative medicine.

Nevertheless, the differentiation process involved is complex and not fully understood. An appreciation of a variety of molecular mechanisms is required in order to enhance protocols that could be replicated for novel therapies. This project has derived ECs from iPSCs using a reliable protocol.

Results here confirmed successful differentiation with an up-regulation in important endothelial markers at the mRNA level alongside positive immunofluorescent staining. These differentiated cells were characteristically similar to mature ECs. In order to expose underlying mechanisms of EC differentiation, the novel gene SETSIP was shown to be upregulated during this process. Further investigation to stimulate differentiating cells with a SETSIP peptide noted an induction in endothelial markers.

Finally, experiments involving epigenetic drugs induced changes in both endothelial marker and SETSIP expression. This project has elucidated the role of SETSIP and data shown here may be important in advancing therapeutic potential through the development of more robust protocols concerning EC differentiation.

Dr Husvinee Sundaramurthi, UCD School of Biomolecular & Biomedical Science, Conway Institute, University College Dublin, Ireland

Identification of novel therapeutic targets for treatment of inherited retinal dystrophies in zebrafish models

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Inherited retinal dystrophies (iRDs) affect 1 in 3000 people worldwide and effective treatment options are not widely available due to the genetic and clinical heterogeneity. Recently, histone deacetylase inhibitors (HDACi) have gained attention as a potential therapeutic option based on their neuroprotective effects within the retina. However, the possible benefits of HDACi remains highly controversial, and their downstream mechanism of action has yet to be thoroughly elucidated. The current study is designed to evaluate the suitability of HDACi to uncover therapeutic options for iRDs using zebrafish models.

A zebrafish retinal mutant model, raifteirí (raf) was selected for the present study and subjected to treatment with two different HDACi, namely tubastatin A (TST) and trichostatin A (TSA), at different time points. At the end of the treatment period, the larvae were subjected to behavioural assays (OKR and VMR), to determine the effect of drug treatment on the visual capacity.

Preliminary data from the study revealed that following TST (selective HDAC 6 inhibitor) treatment, visual function was significantly improved in the treated raf-/-, with an average of 2 saccades/minute ($p = <0.0001$) compared to vehicle control, which had on average 0.2 saccades/minute. Contrarily,
TSA (pan-HDACi) treatment proved to be highly toxic, resulting in adverse effects in the raf line.

Further experiments are needed to determine the maximum tolerated dose, the safety and efficacy profile and the mechanism of action of these drugs. From this pilot study, HDACi have proved to be suitable candidates with potential to uncover additional therapeutic targets for iRDs.

Brandon Tan, School of Medicine, Trinity College Dublin, Dublin 2, Ireland

The risk of QTc prolongation in hospitalized patients on methadone maintenance therapy.

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Introduction: Methadone is a synthetic opioid administered as a heroin substitute. It can result in QTc prolongation on electrocardiogram (ECG) increasing the risk of sudden cardiac death. Methadone is subject to both pharmacokinetic and pharmacodynamic drug-drug interactions which further increase the risk. This study aims to identify inpatients on methadone with and at increased risk of QTc prolongation.

Methods: This prospective study included inpatients on methadone treatment at St James Hospital, Dublin, over a two-week period. The patient's medical chart was reviewed for recorded ECGs. A prolonged QTc was defined as >450ms for males, >470 for females. The electronic patient records were analysed to identify if electrolytes were monitored, if they were abnormal and if they had any comorbities increasing the risk of QT prolongation.

Results: 22 patients from St James's Hospital were included. The mean dose of methadone was 66mg. 68% had an ECG in the chart, 60% of these had a prolonged QTc and 20% had a QTc greater than 500ms. 41% of the group were on drugs with pharmacokinetic interactions with methadone, 28% had pharmacodynamic interactions while 14% had both. Of those with a magnesium level recorded, 41.7% had hypomagnesemia.

Discussion: This study indicated a deficiency in the cardiac monitoring of patients on methadone. The majority of those with an ECG completed had QTc prolongation. The majority were also prescribed medications which increased their risk of a fatal arrhythmia. Prescriber education will improve monitoring of this high risk group and encourage careful prescribing to avoid drug-drug interactions.

Jacinta Walsh, Department Pharmacology & Therapeutics, University College Cork

Host and Environmental Factors Influencing Expression of Bacterial-derived Metabolic Enzymes in Faeces: Potential implications for Microbiota-mediated Drug Metabolism

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The gastrointestinal tract houses a reservoir of bacterial-derived enzymes, including β-glucuronidase and β-glucosidase, that can directly catalyse the metabolism of drugs, for example, β-glucuronidase metabolises Irinotecan, an anticancer drug (1). However, the variation of this metabolic activity across lifespan and experimental species is poorly understood. Therefore, it is important to investigate the host and environmental factors, which may influence the expression and activity of these enzymes, in order to explore novel mechanisms driving inter-individual variation in drug metabolism.

Our aim was to investigate the effects of age, sex, genetic background, germ-free (GF) status, antibiotic treatment, and species on β-glucuronidase and β-glucosidase activity. To quantify the microbial enzymatic activity of the gut microbiome, we prepared an in-vitro metabolism assay, Fecalase; a cell-free extract of faeces.

The absence of enzyme activity in GF animals validated the microbial-derived nature of these enzymes. Our data show that the activity of β-glucuronidase and β-glucosidase depends on sex, age, and species. Additionally, we found that an antibiotic cocktail (vancomycin, ampicillin, and neomycin) administered to mice for 21days significantly impacted enzymatic activity during treatment, which recovered one week after stopping antibiotic administration.

Our data, therefore, suggest that multiple factors can influence the activity of bacterial-derived drug-metabolising enzymes. Moreover, antibiotic treatment can decrease the metabolic activity of the gut microbiota which may have potential implications for drug-drug interactions or cause variations in the efficacy of concomitant medication for several days after finishing an antibiotic course. The implications of these findings for drug metabolism and pharmacokinetics warrant further investigation.

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Rebecca Ward, UCD School of Biomolecular & Biomedical Science, UCD Conway Institute, University College Dublin

Investigation into the underlying aetiology of a visual function defect in a zebrafish model of von-Hippel Lindau disease

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Purpose: Von Hippel-Lindau (VHL) disease is an autosomal dominant disorder affecting 1 in 36000 people. Vision loss and retinal capillary hemangioblastomas remains the most common ocular complications of VHL disease. Van Rooijen et al., (2010) reported retinal and vasculature abnormalities in a recessive zebrafish model of VHL disease. Our aim is to assess vhl-/- visual function, further characterise the ocular defects observed in the vhl-/- eye and pharmacologically rescue vhl-/- function in zebrafish larvae.

Methods: vhl-/- larvae were generated through incrossing vhlhu2117 +/- zebrafish on transgenic background Tg(fli1:EGFP) allowing rapid visualisation of vasculature by fluorescent microscopy.
Larvae were treated at 58 and 72 hpf with Sunitinib and TSA, respectively. Visual behaviour was characterised by optokinetic response (OKR) and visual motor response (VMR) assays. Retinal histology was analysed by bright field microscopy. In addition, intraretinal vasculature was assessed and z-stacks generated by confocal microscopy.

Results: vhl-/- zebrafish display significant loss of visual function at 5 dpf with a >95% reduction in both OKR and VMR, compared to siblings. Abnormal hyaloid vessel development was noted on the lens while ectopic vessels were observed in the retinal layers of 5 dpf larvae. Sunitinib malate significantly improved visual function, reduced intraretinal vessel formation and improved hyaloid vessel patterning. Treatment with Trichostatin A, a HDAC inhibitor was ineffective.

Conclusions: vhl-/- larvae display a visual function defect, hyaloid vessel abnormalities and intraretinal vasculature at 5 dpf which are reversed by treatment with Sunitinib, a tyrosine kinase inhibitor with market approval for kidney, gastrointestinal and pancreatic cancers.

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Elucidating the role of anaesthetic/analgesic target receptors in breast cancer metastasis.

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Breast cancer-related mortality is due to the burden of metastatic disease. The peri-operative period is a critical time in metastatic development and perioperative factors, including anaesthetic /analgesic drugs, may influence cancer outcome. We previously identified nine anaesthetic/analgesic receptor targets whose expression is associated with clinical metastasis in breast cancer. Here, we aimed to provide a functional basis for these observations by investigating receptor-mediated mechanisms by which analgesic/anaesthetic drugs influence metastasis.

Migration on collagen was determined using the OrisTM Cell Migration Assay and invasion was measured using a three-dimensional, hanging-drop spheroid assay. Protein expression was determined by immunoblotting with densitometry.

The migratory potential of five human breast carcinoma cell lines was: MCF7 > CAL51 > MDA-MB-231 > BT-549 > MDA-MB-468 and their invasive potential was: MDA-MB-231> BT549 ≈ MCF7 > CAL51 >>> MDA-MB-468. Five of the nine receptors displayed differential expression patterns in these cell lines. GABARγ3 and glycine receptor β were expressed most prominently in the most migratory MCF-7 and CAL-51 cells. Noradrenaline transporter protein, NET, and opioid receptor μ were strongly expressed in the poorly migratory MDA-MB-468 cells. Opioid receptor δ was prominently expressed in MCF-7, CAL-51 and MDA-MB-468 vs MDA-MB-231 and BT-549 cells.

Acute (3-h; 500nM) exposure to morphine significantly increased MCF-7 migration at 48h and this was reversed by the selective mu and delta opioid receptor antagonists cyprodime and naltrindole. Similar effects were observed in MDA-MB-231 cells. Acute exposure to glycine (3h; 250uM) decreased MDA-MB-231 invasion and this was reversed by the glycine beta receptor antagonist strychnine.