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Establishment of a photothrombotic stroke model in rats for investigations into therapies for improving recovery after stroke. **L Boddington, J Gray, J Reynolds. Brain Health Research Centre, Department of Anatomy, Otago School of Medical Sciences, University of Otago, Dunedin.**

Stroke is a leading cause of disability with many survivors showing some degree of motor impairment. After a stroke the brain undergoes remapping, allowing limited recovery of lost motor function. However, recovery is potentially hindered by changes in neuronal excitability. This study set out to establish a photothrombotic stroke model in rats to be used for future investigations into therapies to improve recovery after stroke.

Photothrombotic lesions were induced in the motor cortex of three rats. The photosensitive dye 'Rose Bengal' was injected intravenously and the motor cortex illuminated with intense light for 30 minutes to induce local platelet aggregation and thrombus formation. Another three rats underwent stroke induction surgery and light exposure, however no lesion was induced (unlesioned shams). Deficits in forelimb co-ordination were assessed using a grid-walking task and any asymmetry in exploratory forelimb use was detected using a cylinder task.

Before stroke induction, no significant asymmetry in forelimb use was observed, however after surgery, lesioned rats showed a persistent significant bias in using their unaffected forelimb (61.0% ± 5.9, mean ± SD, $P < 0.05$, One-way ANOVA). Unlesioned sham rats showed no significant bias in paw use after surgery. Prior to surgery, rats showed good co-ordination when walking across a wire grid, with less than 3% of the total steps taken being considered as stepping errors. During the first week after stroke induction, stepping errors in lesioned rats rose significantly (9.9% ± 3.6, mean ± SD, $P < 0.05$) compared to baseline assessments, but recovered to baseline levels after four weeks. Unlesioned rats showed no significant change in stepping errors over the course of the study.

Establishment of this reproducible model of stroke and functional deficit in rats will allow for future studies of the application of stimulation protocols thought to improve functional recovery after a stroke.

Nanomicelle-encapsulated RL71 (SMA-RL71) retains cytotoxicity against estrogen receptor negative breast cancer cell lines. **J Diong[1], R Rosengren[1], L Larsen[2], K Greish[1]. [1]Department of Pharmacology and Toxicology, Otago School of Medical Sciences, [2]Department of Chemistry, Division of Sciences, University of Otago, Dunedin.**

Triple negative breast cancer (TNBC) is an aggressive subset of breast cancer lacking targeted drug treatments. The novel drug RL71 is cytotoxic toward TNBC cell lines

with submicromolar EC50 values. To effectively deliver RL71 utilising the enhanced permeability and retention (EPR) effect, this study aimed to optimally encapsulate RL71 into styrene-maleic acid micelles (SMA-RL71) and examine the *in vitro* cytotoxicity of SMA-RL71 towards estrogen receptor negative breast cancer cell lines.

To synthesise micelles, RL71 and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide were added to hydrolysed styrene-maleic acid. Micelles were precipitated at pH 5, centrifuged, solubilised in water at pH 11 and then neutralised. The release rate of RL71 from micelles was determined by dialysis in a water bath at pH 7.4 and pH 5.5. The size of micelles was established using dynamic light scattering. MDA-MB-231, MDA-MB-468, Hs578T and SKBr3 cells were treated with RL71 or SMA-RL71 (5% or 15% loading).

Synthesised micelles had diameters of 130 nm (5% loading) and 182 nm (15% loading) which were large enough to utilise the EPR effect. In tumour-like conditions at pH 5.5, 24.5% ± 0.14% and 8.28% ± 0.7% (5% and 15% loading, respectively; mean ± SEM, n = 3) of RL71 was released from micelles after 6 hours. SMA-RL71 (15% loading) was the most cytotoxic towards MDA-MB-231 cells (EC50 of 0.54 µM compared with 0.78 µM for free RL71). SMA-RL71 (15% loading) was also more cytotoxic than RL71 towards MDA-MB468 and Hs578T cells, with EC50 values of 0.98 µM and 0.61 µM, respectively (compared with 1.05 µM and 0.88 µM for RL71). SMA-RL71 (5% loading) was less cytotoxic than RL71 and SMA-RL71 (15% loading) in all cell lines.

These results indicate that cytotoxicity was maintained for both constructs of SMA-RL71, largely in the submicromolar range. Furthermore, the micelles possessed desirable characteristics for further *in vivo* examination.

Changes in rat supraoptic nucleus gene expression in pregnancy and lactation. A Seymour, R Augustine, V Scott, C Brown. Centre for Neuroendocrinology and Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin.

Birth and lactation are partly controlled by the hormone oxytocin, which is synthesised in the hypothalamic supraoptic nucleus (SON) of the brain. This study used quantitative PCR to investigate changes in gene expression of *oxytocin, c-Fos, JunB* and *prodynorphin* at different stages of reproduction to determine whether these might be involved in the driving activity of the oxytocin system in pregnancy and lactation.

Conscious rats that were either non-pregnant diestrous (NP, n = 8), pregnant at day 7 (P7, n = 6), 14 (P14, n = 6), or 21 (P21, n = 7), lactating at day 7 (L7, n = 5) or post-weaning (PW, n = 4) were euthanised by decapitation. Micropunches were taken from the SON and RNA was extracted and reverse-transcribed to cDNA that was then amplified using primers specific for the gene of interest. Gene expression was calculated relative to the housekeeping gene β-actin and expressed as a percentage of NP levels.

SON *oxytocin* gene expression was not different between groups ($P = 0.18$, one-way ANOVA). Similarly, SON *c-Fos* expression was not different between groups ($P =$

0.29) By contrast, SON *JunB* expression was different between groups ($P = 0.02$), as was SON *prodynorphin* expression ($P < 0.01$). SON *JunB* expression was lower than NP at P7 (61.8 ± 8.6% of NP, mean ± SEM, $P < 0.05$, Dunnett's *post-hoc* test), P14 (55.6 ± 5.1%, $P < 0.05$) and L7 (58.5 ± 5.4%, $P < 0.05$). SON *prodynorphin* expression was higher at L7 than NP (235.1 ± 67.9% of NP, $P < 0.05$).

The results from this study provide evidence that decreased *JunB* expression and increased *prodynorphin* expression might be associated with altered functioning of the oxytocin system in pregnancy and lactation.

Microbiological quality of imported sexual performance medications. **W Shen[1], S Mros[2], M McConnell[2], S Hook[1], C Strachan[1]. [1]School of Pharmacy, [2]Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago, Dunedin.**

Counterfeit medicines, adulterated natural products and invalidly imported prescription medicines are examples of importations that are seized by the border control pharmacist. While the pharmaceutical quality of such products have been found to be sub-standard, there is a lack of appreciable data on the microbiological quality of these imported products, usually purchased via the internet. This study therefore examined the microbiological quality of confiscated medicines and natural products produced for aiding sexual performance, which make up a large proportion of the items seen and detained by border control.

The products provided by border control included: counterfeit Viagra® (sildenafil), counterfeit Cialis® (tadalafil), three generic sildenafil medications and seven natural products. Additionally, four natural products were purchased locally, as well as genuine Viagra® and Cialis®. Testing methods were based on those given by the European Pharmacopoeia. Product contamination was quantified by the enumeration of total aerobic micro-organisms, yeast and moulds, and bile-tolerant, Gram-negative bacteria. Absence/presence testing for faecal coliforms, *Salmonella* spp., and *Clostridium* spp. were carried out by enriching then sub-culturing onto differential agars. Results were then compared to pharmacopoeial acceptance criteria.

Seventeen of the 18 products tested conformed to pharmacopoeial standards. One of the imported natural products recorded an aerobic microbial count of 5.74×10^5 colony-forming units per gram of product (CFU/g), exceeding the pharmacopoeial limit of 1.0×10^5 CFU/g for herbal products. The presence of *Enterobacter* and *Clostridium* were detected following enrichment, suggesting possible faecal contamination. No faecal coliforms should be present in oral dosage forms for human use.

This is the first study to examine the microbiological quality of confiscated imported medicinal products in New Zealand. While it is pleasing to note that all but one of the products were of an acceptable microbiological quality, the pharmaceutical quality of these products, particularly those claiming to be natural, should be examined.

"Mummy, Are There Bugs in My Mouth?": Investigating the Oral Bacterial Diversity of Dunedin Children. **D Sundaresan[1], M Cullinan[1], B Drummond[1], J Stanton[2], G Seymour[1], N Heng[1]. [1]Sir John Walsh Research Institute, Faculty of Dentistry, [2]Department of Anatomy, Otago School of Medical Sciences, University of Otago, Dunedin.**

The adult oral cavity is thought to harbour > 700 microbial species, some of which cause oral diseases such as periodontitis. However, little is known about the number and types of oral species present during a child's dental development. This project aimed to characterise the oral bacterial diversity of children at key stages of dental development using next-generation DNA sequencing technology and bioinformatics.

Samples were taken from the teeth of 12 dentally-healthy children across four age groups ranging from 10 months to 7 years. A fifth group (3 children, aged 4 - 5) with confirmed active caries lesions was also included. The highly-conserved bacterial 16S rRNA genes were PCR-amplified from each sample and sequenced using the GS-FLX Titanium pyrosequencer. All sequence data was processed using the CLOTU (CLuster Operational Taxonomic Unit) bioinformatics suite.

Analysis of the bacterial diversity in all of the children's dental samples revealed only 40 to 128 distinct species. At 10 - 12 months of age, bacteria on the teeth are predominantly Gram-positive with 35% - 46% of sequences classified as *Streptococcus* spp. and *Abiotrophia*. In contrast, at 6 - 7 years, dental surfaces comprise mainly Gram-negative taxa including *Leptotrichia* (~25%) and *Fusobacterium* (~10%). Genera such as *Rothia* were found in comparatively constant proportions (~10%) across all age groups. Interestingly, higher levels of *Streptococcus sanguinis* (~19%), and not the expected *Streptococcus mutans*, were detected in children with active caries relative to their age-matched dentally-healthy counterparts.

In summary, despite an overall limited range of species, age-dependent shifts in oral bacterial diversity were evident at different stages of a child's life. The consistent presence of under-studied genera such as *Leptotrichia* and *Rothia*, and the prominence of *S. sanguinis* in carious samples, demonstrate the need for further research into their respective roles as potential microbial markers of oral health status.

Delayed post-treatment with bone marrow-derived mesenchymal stem cells affects the proliferation of progenitor cells in the subventricular zone after neonatal rat hypoxic-ischemic brain injury. **S Cameron, L Goddard, R Sizemore, D Oorschot. Department of Anatomy, Otago School of Medical Sciences, University of Otago, Dunedin.**

Hypoxic/ischemic (H/I) brain injury is a major contributor to neurodevelopmental deficits such as cerebral palsy. Striatal medium-spiny neurons die after this injury. The absolute number of striatal medium-spiny neurons is restored at one week after delayed treatment with bone marrow-derived mesenchymal stem cells (MSCs). The

adjacent subventricular zone (SVZ) is a source of progenitor cells that may restore these neuronal numbers. Whether treatment with exogenous MSCs facilitates neurorestoration via progenitor cell proliferation, migration, survival and differentiation is unknown. Hence, we investigated whether MSCs affect progenitor cell proliferation in the SVZ after neonatal hypoxia-ischemia.

Postnatal day (PN) 7 male Sprague Dawley rat pups underwent ligation of the right common carotid artery followed by exposure to 8% oxygen/92% nitrogen for 1.5 h. On PN14 a subcutaneous injection of cultured bone marrow-derived MSCs (126,000 cells), sourced from rat femurs, or diluent (saline) was administered to four H/I rats, respectively. Animals were perfused on PN21, each cerebrum was serially sectioned, and the sections processed for immunohistochemistry. The primary antibody MIB-5 raised against Ki-67, a specific marker of cellular proliferation, was utilized. The primary antibody was detected using biotinylated secondary and streptavidin-peroxidase antibodies, with aminoethylcarbazole as the end label. Sterelogical methods were used to measure the absolute number of Ki-67-positive cells in the SVZ.

There was a statistically significant reduction in the absolute number of Ki-67-positive cells in the SVZ of H/I animals treated with MSCs (1380 ± 120, mean ± SEM, n = 3) compared to H/I diluent-treated animals at one week after treatment (3390 ± 537, n = 4, unpaired two-tailed Student's *t*-test; $p = 0.03$). This decrease in the number of proliferating progenitors in the SVZ suggests that MSCs may be effective in stimulating the migration, survival and differentiation of SVZ progenitor cells into nearby striatal neurons. This is currently being investigated.

Upregulation of chemokine expression is associated with oestrogen deprivation in ER+ breast cancer. B Hunter, A Dunbier. Department of Biochemistry, Otago School of Medical Sciences, University of Otago, Dunedin.

Breast cancer is the most common cancer amongst women and the leading cause of cancer-mortality in females, with particularly high incidence amongst those living in developed countries. Approximately 80% of breast cancers express oestrogen receptor-□ (ER) and use oestrogen as a key growth stimulus. Aromatase inhibitors (AIs) reduce tumour cell proliferation by blocking the production of oestrogen. Despite this, up to 50% of patients receive little to no benefit from this therapy and many ultimately relapse. Treatment failure has recently been found to be associated with increased immune cell infiltration and inflammatory gene expression. Chemokine production by tumour cells represents one mechanism through which immune cells could be recruited. This study aimed to investigate the effect of oestrogen deprivation on expression of chemokines in the human cancer-cell line MCF-7 and in patients treated with AIs.

To mimic AI therapy, MCF-7 cells were cultured for 5 days in steroid-stripped fetal bovine serum and chemokine expression was measured at days 0, 1, 3 and 5 using quantitative real-time PCR. Chemokine expression was measured relative to the housekeeper genes FKBP, PUM1 and TPB that are known not to change during oestrogen deprivation.

Oestrogen deprivation induced cells to up-regulate expression of *CCL5*, *CCL22* (8-fold and 9-fold of control cultures respectively $P < 0.05$, unpaired two-tailed t-test) and *CXCL16* (2-fold, P = ns) after 3 days. Bioinformatic analysis of gene expression data from AI-treated patients revealed up-regulation of *CCL5* and *CXCL16* ($P < 0.05$). Genetic data was derived from patients involved in two clinical trials by Dunbier *et al.* and Miller *et al.*. High pre-treatment expression of *CXCL16* and *CXCL14* were also associated with increased survival.

These data suggest that oestrogen deprivation induces chemokine expression in ER+ve tumour cells *in vitro* and *in vivo*. Chemokines have the potential to recruit immune cells that could facilitate tumour growth in the absence of oestrogen. Targeting these cells could provide a novel therapeutic approach to improve response to treatment.

Does the schizophrenia-inducing cytokine IL-6 alter neurite outgrowth in the developing brain? S Murray, C Jasoni. Department of Anatomy, Otago School of Medical Sciences, University of Otago, Dunedin.

Maternal infection during pregnancy is a risk factor for schizophrenia. Maternal immune activation (MIA) in rodent models causes up-regulation of cytokines that can access the fetus, leading to schizophrenia-like behaviours in adult offspring. Pro-inflammatory cytokines, especially Interleukin-6 (IL-6), are strongly implicated in the development of this schizophrenic phenotype, however little is known about what effect cytokines may be having in the brain during development. The growth of neurites and formation of neuronal connections are essential for development of functional neural circuits in the brain. Therefore, the present study aimed to investigate the effect of IL-6 on neurite outgrowth in the developing hippocampus.

Hippocampi obtained from gestational day 17.5 (GD17.5) and postnatal day 0 (P0) mice, were cut into 300µm explants and plated in a 3D collagen gel containing either 10 ng/ml IL-6 or vehicle (phosphate buffered saline). The plated explants were incubated for 24 or 48 hours, then fixed and stained with the neuron-specific antibody TuJ1. Images were obtained using confocal microscopy and analyzed using computer software ImageJ. A ratio of outgrowth was calculated using the number of pixels outside the explant divided by the number of pixels inside the explant.

Neurite outgrowth in the treated group was expressed as a percentage of outgrowth in the control group. At P0, outgrowth of IL-6 treated explants (n = 6 explants) was 95.9% of control outgrowth (n = 5) at 24 hours, however at 48 hours outgrowth in the treated group (n = 21) was significantly reduced to 35.3% of control outgrowth (n = 15, $P < 0.05$, Student's *t*-test). At GD17.5, outgrowth of IL-6 treated explants was not significantly different from controls at either 24 or 48 hours.

These results suggest that exposure to elevated IL-6 found in MIA can disrupt hippocampal wiring, forming a mechanism for schizophrenia risk in offspring.

Reduced cardiac response to β-adrenergic stimulation in the diabetic rat heart. **H-Y Wang[1], J Baldi[2], R Lamberts[1]. [1]Department of Physiology, Otago School of Medical Sciences, [2]Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin.**

Diabetes mellitus has a profound detrimental effect on the heart, with diabetic patients having a greater risk in the development of cardiovascular complications during and after surgery. It is established that the diabetic myocardium has a reduced cardiac response to β-adrenoceptor (β-AR) stimulation by catecholamines, but whether this is due to decreased inotropy (contractile force), chronotropy (heart rate) or both is unknown. Therefore, this study aimed to determine whether myocardial inotropic and chronotropic responses to β-AR stimulation are reduced in diabetes, using the isolated hearts of Zucker-diabetic-fatty (ZDF) rats.

Langendorff-perfused hearts of non-diabetic and diabetic ZDF rats (male, 16 weeks, n = 8) were randomly assigned to paced (5 Hz) or unpaced (intrinsic) heart rate. After basal functional parameters were measured, the hearts were exposed to incremental concentrations of dobutamine (10^{-9} to 10^{-6} M), a β-AR agonist. Left ventricular pressure (P_{LV}) and intrinsic heart rate (HR) were measured to assess inotropic and chronotropic response, respectively.

In the paced hearts, no change in basal P_{LV} was observed. In unpaced hearts, a depression in basal HR was detected in diabetic compared to non-diabetic (250 ± 15 versus 178 ± 9 bpm, $P < 0.05$), whereas the proportional HR increase in dobutamine concentrations was similar in both groups. There was a reduced response in P_{LV} to incrementing dobutamine concentrations in the diabetic group, which was more pronounced in the unpaced heart (ΔEC_{50} between non-diabetic and diabetic group: 0.47×10^{-7} M paced versus 1.76×10^{-7} M unpaced, $P < 0.05$).

The reduction in basal heart rate and compromised inotropic response to catecholamine in the diabetic heart is likely to be caused by the sympathetic overdrive present in diabetic rats *in vivo*. Although chronotropic modulation by dobutamine is unaltered, the HR-dependent inotropic response shows a reduced association between HR and contractility (Treppe effect) in the diabetic heart.

Apoptotic cell death in the rat hippocampus following a single binge alcohol exposure. **C Smith[1,2], L Fisher[2], R Napper[1,2]. [1]Brain Health Research Centre, [2]Department of Anatomy, Otago School of Medical Sciences, University of Otago, Dunedin.**

Drinking alcohol during pregnancy can result in profound developmental defects in the child, especially in learning and memory. Animal studies show that apoptotic cell death occurs in the developing brain soon after alcohol exposure. During apoptotic degradation, the contents of the cell are destroyed, presenting a challenge in determining which cell type is dying. Many of the neuronal populations vulnerable to ethanol toxicity are postmitotic and neuronal deficits will result in changes in brain circuitry. A deficit of hippocampal CA1 neurons in mature animals, exposed to alcohol on postnatal day 6 (PN6) suggests acute CA1 neuronal death. This study

investigated the time interval during which apoptotic cells can be phenotyped after the initiation of apoptosis.

Alcohol was delivered to rat pups on PN6, as two gavages of alcohol, administered two hours apart. A high dose (6 g/kg body weight) was used to achieve a high blood alcohol concentration (greater than 400 mg/dl). Animals were sacrificed 4, 8, and 12 hours after the initial alcohol exposure. Tissue sections were labelled using immunohistochemistry methods to detect astrocytes (GFAP/Vimentin), neurons (NeuN), and apoptotic cells (activated caspase-3 and Hoescht 55542).

The results showed an increase in apoptotic cell death in hippocampal sections between 4 to 12 hours (from 56 ± 13 to 246 ± 32 respectively). Maximal co-localisation of NeuN/activated caspase-3 occurred at 4 hours, with a significant decrease in NeuN/activated caspase-3 from 4 to 12 hours (57.29% ± 1.94% to 11.91% ± 1.73% respectively, $P < 0.001$). Activated caspase-3/Hoescht co-localisation was high at all time points (100% at 4 hours, 93.45% ± 2.61% at 8 hours, and 87.78% ± 1.03% at 12 hours). Data was analysed using one-way ANOVA and Newman-Keuls post-hoc test (Graphpad Prism).

The results confirm that CA1 neurons undergo apoptotic cell death following ethanol exposure in the CA1 region. Phenotypic identification is optimal at 4 hours after activation of the apoptotic cascade.