

Poster Presentation

Presenter: Andrew Bondoc

Use of a luciferase reporter transgenic murine line to target cardiac stem cells following implantation into mouse models of Duchenne muscular dystrophy

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Area of Research: Cell Biology

Background: Duchene muscular dystrophy (DMD) is an x-linked neuromuscular disorder characterized by progressive muscle deterioration affecting skeletal and cardiac muscle. Over 95% of DMD patients suffer from cardiomyopathy, and up to one-third of DMD deaths are attributed to heart disease. Transplantation of resident cardiac stem/progenitor cells from healthy individuals is a promising therapeutic approach to DMD-associated cardiomyopathy. To make research of this novel therapeutic strategy feasible, a non-invasive method of assessing treatment efficacy must be devised in a model organism.

Rationale: Coupling bioluminescence reporter gene firefly luciferase (Luc) to cardiomyocyte-specific promoter alpha myosin heavy chain (α MHC) and generating a transgenic C57BL/6 mouse line harboring this gene will allow monitoring of transplanted cell engraftment, long-term survival, and differentiation in DMD models.

Objective: To characterize the α MHC-Luc transgenic murine line

Methods: 1) genotyping via PCR analysis on tail snips was conducted to identify transgene-positive adult mice; 2) tissue-specific expression of the Luc reporter in newborn and adult mice was assessed using western blot analysis and immunohistochemistry (IHC); 3) cardiac stem/progenitor cells were FACS-sorted, and their ability to differentiate into cardiomyocytes demonstrated via immunocytochemistry (ICC); 4) sorted cells were implanted into dystrophic mice and targeted via bioluminescence imaging and IHC to correlate.

Results: Transgene-positive newborn mice did not express Luc in the myocardia. However positive adult mice showed signs of transgene expression in heart tissue, possibly due to differential gene expression in the adult stage. Differentiable cardiomyocytes were traceable via IHC to a limited extent.

Future Direction: Cardiac stem/progenitor cells harvested from α MHC-Luc mice will serve as a source of cells to be implanted into the myocardia of dystrophic mice for further imaging research.

Poster Presentation

Presenter: Katherine Cheung

Intrathecal Baclofen in Patients with Spasticity Caused by Spinal Cord Injury: A Systematic Review

Kung Yan Cheung¹, Amanda McIntyre, MSc¹, Swati Mehta, BSc¹, Robert W. Teasell, MD FRCPC^{1,2}, Dalton L. Wolfe, PhD^{1,2}, and the SCIRE Research Group

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Purpose: To systematically review the effectiveness of intrathecal baclofen in reducing spasticity for people with spinal cord injury (SCI).

Relevance: Complications relating to spasticity are experienced by 53% to 78 % of individuals with SCI. Of the currently available treatments, intrathecal baclofen is a commonly used management technique to reduce spasticity in SCI patients.

Methods: A key term literature search was conducted in the following electronic databases: Medline, CINAHL, EMBASE, and PsycInfo. Key words used for the literature search included spinal cord injury, tetraplegia, paraplegia, bolus, intrathecal baclofen, and spasticity. Retrieved references were also scanned for other missing, relevant citations. Studies published before October 2011 were evaluated and included if the study involved an intervention examining the use of intrathecal baclofen in reducing spasticity post SCI.

Study Sample: Upon review of 115 titles and abstracts, 11 studies (2 level 1 studies; 9 level 4 studies) met the inclusion criteria. Pooled sample size of all included studies was 323. Sample sizes of each study ranged from 7 to 75 with a mean of 29. Mean follow up period of each study was 26.6 months.

Findings: In general, studies found a significant outcome reduction in spasticity measures ($p < 0.005$). Nine studies that used the Ashworth scale as an outcome measure found a mean improvement greater than 2 points on the 5 point scale ($p < 0.05$). Similarly, six of the seven studies using the Penn spasm frequency scale found an improvement in mean score greater than 2 points ($p < 0.05$). Several level 4 studies noted functional improvements in areas such as self-care, wheelchair transfer and personal hygiene. Five studies that included tolerance measure noted a dosage increase from an initial mean range of 83.2 - 373 $\mu\text{g}/\text{d}$ to a final mean range of 193-567 $\mu\text{g}/\text{d}$ after the initial 12 month study period. One study observed that dosage did not change over time. Common adverse effects included: catheter dislocation, seroma, infections and orthostatic hypotension.

Conclusion: Intrathecal baclofen is highly effective in reducing spasticity for individuals post SCI. Side effects were reported to be low to moderate.

Support: This project was supported by the Rick Hansen Institute and Ontario Neurotrauma Foundation.

Poster Presentation

Presenter: Katherine Cheung

A Systematic Review on the Relationship between Bladder Management Techniques and Quality of Life Following Spinal Cord Injury

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Purpose: To systematically review the effectiveness of bladder management techniques in improving quality of life (QoL) for people with a spinal cord injury (SCI).

Relevance: 81% of people with SCI report having bladder dysfunction. Studies have suggested that the choice of an inappropriate bladder management method may negatively affect patient's QoL. This systematic review is conducted to examine the relation between neurogenic bladder management techniques and QoL post-SCI.

Methods: A key terms literature search was conducted in the following electronic databases: Medline, CINAHL, EMBASE, and PsycInfo. Studies published before September 2011 were reviewed and evaluated based on the following criteria 1) at least three study participants; 2) at least 50% of participants had an SCI; and 3) study subjects participated in a bladder management intervention and included an assessment of QoL.

Study Sample: Upon review of 367 titles and abstracts, 11 studies (2 level 1 RCT; 5 level 2 studies; 4 level 4 studies) met the inclusion criteria. Pooled sample size of all included studies was 728. Sample sizes of each study ranged from 7 to 142 with a mean of 66.

Findings: Studies examined three approaches for bladder management and/or treatments: conservative bladder emptying (n=5) including intermittent or indwelling catheterization, electrical stimulation (n=3), and botulinum toxin treatment (n=3). Of all the treatments, 300U of botulinum toxin treatment was shown to be most effective in improving quality of life, supported by two RCTs of level 1 evidence ($p < 0.001$ and $p \leq 0.002$ respectively). Electrical stimulation via the Brindley stimulator was also shown to be advantageous in improving QoL ($p = 0.046$). Most studies found a strong relationship between a reduction in incontinence episodes, an improvement in bladder functioning, and an increase in quality of life.

Discussion: Regarding QoL, there is a paucity of evidence supporting which bladder management treatment provides the greatest improvement. The decision to use any given treatment is likely multifactorial and independent in nature. This decision will need to consider biological and physiological conditions, as well as safety, accessibility, and practicality.

Conclusion: This review has suggested that positive changes in incontinence episodes and improvement of bladder functioning were the strongest influencing factors in increasing QoL among people with SCI attempting to manage their bladder.

Support: This project was supported by the Rick Hansen Institute and Ontario Neurotrauma Foundation.

Poster Presentation

Presenter: Michael Ruiz

Investigating the Involvement of Endothelial Derived MicroRNA-200b to Angiogenic and Inflammatory Processes in Diabetic Nephropathy

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Epigenetic regulations like microRNA are showing increasing role in the pathogenesis of diseases like diabetes. Our lab has previously described microRNA-200b (miR-200b), a post-transcriptional regulator of vascular endothelial growth factor (VEGF) and other factors, in the context of diabetic retinopathy. However, miR-200b has yet to be studied in diabetic nephropathy. Human umbilical vein endothelial cells were incubated for 24 hours in low glucose (5mM) and high glucose (25mM). RNA was isolated using trizochloroform method and miR-200b, VEGF mRNA and p300 mRNA was quantified by RT-PCR. MiR-200 was found to be downregulated, with consequent over expression of VEGF and p300 factors involved in angiogenic and fibrotic mechanisms of diabetic nephropathy. A future experiment is proposed that will utilize a transgenic mouse strain we have created with the help of London Regional Transgenic and Gene Targeting Facility. The goal of the proposed experiment is to investigate the effects of miR-200b over expression selectively in endothelial cells to elucidate the signaling relationships and significance between these factors. MicroRNA present an interesting research subject due to their potential to effect a multitude of targets with a cell very specifically. The field of microRNAs is quickly growing, so investigating targets like miR-200b provides more understanding into the signaling events involved in diabetic nephropathy. With such knowledge comes the potential to better understand out underlying physiology, as well contribute to the development of novel treatments.

Poster Presentation

Presenter: Imran Schabeer

Immunohistochemical Analysis of Gastroenteropancreatic Neuroendocrine Tumors: A Tissue MicroArray Study

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Currently there are no accurate and well-characterized biomarkers that can be routinely used in the clinic to characterize Gastroenteropancreatic Neuroendocrine Tumours (GEP NETs) and guide subsequent management of these lesions. This is largely because our understanding of the molecular and cellular biology of these tumours is limited. As a result, we have also seen little improvement in the management of these lesions in the last few decades. The present study is aimed at using high through-put immunohistochemical staining to investigate the potential of various proteins to be used as prognostic biomarker in predicting tumour behaviour of GEP NETs.

Tissue Microarrays (TMA) will be constructed and immunohistochemical staining will be subsequently employed to compare protein expression patterns between two main experimental groups: primary GEP NETs and GEP NETS metastases to liver. Binary comparisons will be made between primary GEP NETs and GEP NETs metastatic to the liver to identify any significant correlations between protein expression and tumour grade. Our use of TMA will allow for high-throughput molecular profiling of our tumour specimens.

We expect to find a few proteins with expression profiles that have significant correlation with the malignant potential of a tumour. These expression patterns would indicate their potential in predicting tumour behaviour in GEP NETs. In addition, our findings will contribute to the better understanding of the molecular biology of these tumours by identifying proteins that are affected in the pathogenesis of this tumour. The study presents a first step in discovering and potentially establishing various biomarkers that can accurately and effectively predict behavior in GEP NETs and, subsequently, aid in better management of these lesions.

Oral Presentation

Presenter: Adrian Chan

Human HERCs: a novel family of cellular HIV-1 restriction factors.

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Cellular HIV-1 restriction factors are interferon-induced proteins that inhibit different stages of the HIV-1 lifecycle, thereby blocking HIV-1 replication. We recently identified a new HIV-1 restriction factor called HERC5 (HECT domain and RCC1-like domain-containing protein 5) that blocks HIV-1 assembly at a late stage of the HIV-1 lifecycle. This inhibition was a result of the E3 ligase activity of HERC5, which is responsible for conjugating a small protein called interferon stimulated gene 15 (ISG15) onto a key structural protein of HIV-1 called Gag. HERC5 belongs to the *herc* gene family, of which there are six members (*herc1* to *herc6*) and can be further sub-divided into large (HERC1 and HERC2) and small (HERC3-6) HERCs. The small HERCs all possess a similar domain architecture of one RCC1-like domain, a Spacer region, and one HECT domain. The anti-HIV activity of the small HERC members HERC3, HERC4 and HERC6 was previously unknown. Given the conserved nature of the small HERCs, we hypothesized that *herc3*, *herc4*, and *herc6* could inhibit HIV-1 replication, similar to HERC5. We co-transfected a plasmid encoding *herc3*, *herc4*, *herc5*, or *herc6* with a plasmid encoding the HIV-1 genome into HeLa cells. After transfection, HIV-1-particles released into the supernatant were harvested and separated by SDS-PAGE. Western blot analysis revealed that *herc4*, *herc5* and *herc6* all inhibited HIV-1 replication, as shown by the lack of HIV-1 particles in the cell pellet and cell supernatant. However, *herc3* was unable to inhibit HIV-1 replication. In conclusion, the small HERC proteins represent a novel family of potential cellular restriction factors, highlighting a potential new avenue for HIV/AIDS therapeutics.

Oral Presentation

Presenter: Kevin Cheung

Immunohistochemical analysis of protein markers in mice with experimental allergic encephalomyelitis induced by MOG(35-55) peptide and in humans with multiple sclerosis.

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Multiple sclerosis (MS) is a neurodegenerative disease involving focal demyelination and axonal damage in the central nervous system. Shown to have a strong autoimmune component, MS treatment options involve suppressing the immune system, though this is not curative and only serves to slow its progression. Angiogenesis is also believed to play a part in MS, but its importance and role in the disease has not been fully explored. This study examines possible protein factors involved in the pathogenesis of MS. The proteins chosen for study were based on research performed earlier in our lab by Roscoe et al. (2009). Using microarray analysis on mouse spinal cords with experimental allergic encephalomyelitis (EAE), the primary animal model of MS, several proteins were found to be increased. The specific proteins chosen for study were: Laminin, VEGF, Iba-1, HIF-1 alpha, Caspase 8. To analyze protein expression, immunohistochemistry was utilized on human MS spinal cord and brain tissue. In addition, histological staining with Hematoxylin & Eosin and Solochrome R cyanine was used to determine lesion location. Equivalent staining methods were performed on mouse EAE spinal cords for comparison purposes. To observe the staining a light microscope was used and every stain was given a pathological score based on a pre-defined scoring scale from Roscoe et al. (2009). Spearman correlation in rank order and linear regression analysis was performed on the spinal cord data; the brain data is pending. Our observations show a strong relationship between increased expression of all protein factors and lesion appearance in human MS tissue. Our data shows, significantly, that laminin is highly correlated with demyelination ($n = 9$; $p < 0.05$; correlation coefficient = 0.833; $R = 0.849$). We interpret this result as higher vessel numbers appear when higher lesion numbers are observed, and these findings support the need for further study on the angiogenic aspect of MS.

Oral Presentation

Presenter: Cameron Goertzen

Also Presented Poster Presentation

Digital Quantitative Pathology of Carotid Atheromas: 3D Correlative Studies with Ultrasound, PET/CT and MRI.

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The purpose of this study was to determine carotid atheroma histology for comparison with pre- and post-operative images created with Ultrasound, PET/CT and MRI. We hypothesized that digital analysis is feasible, reliable, and can improve the sensitivity and specificity of current imaging modalities in the identification of atheromatous pathology. Patients have been recruited from stroke clinics in London, Ottawa and Toronto and have undergone pre-operative imaging of their carotid artery using 3D ultrasound and PET/CT scans. Plaques were removed by carotid endarterectomy, imaged with MRI and micro CT, sectioned and stained, then converted to digital images. Using Aperio's Image Scope, the digital images have undergone manual and semi-automated analysis to produce histological annotations. Kappa analysis was performed on five representative slides containing the histological feature of interest to determine intraobserver and interobserver agreement. To date we have analyzed four patients totaling 132 sections. Quantitatively, we have determined total area of calcification, lipid core, hemorrhage, hemosiderin, inflammation and neovascularization as well as average fibrous cap thickness. Qualitatively, we have determined incidence of thrombus, probable ulceration, and probable plaque rupture. Kappa analysis has revealed adequate intraobserver agreement for calcification (K=0.9091), lipid core (K=0.8872), hemorrhage (K=0.8026), neovascularization (K=0.9248) and hemosiderin (K= 0.6582). Interobserver kappa analysis has also resulted in adequate agreement (K= 0.8593). Due to the low incidence of qualitative features, analysis of more patients must be performed in order to gather sufficient data to determine representative kappa scores. Using the quantitative data, we will now correlate with the clinical and radiological findings in expectation that this will increase accuracy and resolution of current imaging modalities. By enhancing screening techniques, clinical triage will be improved as well as the ability in identifying patients who should undergo carotid endarterectomy surgery.

Oral Presentation

Presenter: Cecilia Kwok
Also Presented Poster Presentation

Rectreation Drug and Alcohol Use Among Individuals With Acquired Brain Injury Attending Inpatient rehabilitation

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Introduction: It is not uncommon for individuals who have sustained a traumatic brain injury to have had a history of substance abuse. Quite often recreational drugs and alcohol are consumed just prior to their injuries. Substance use can have a considerable impact on the recovery of a person with an acquired brain injury (ABI), and their overall functioning later in life.

Methods: A retrospective chart audit was conducted on 102 patients admitted to a neurorehabilitation unit in London, Ontario between March 2009 and March 2011 to determine the prevalence of substance abuse among individuals with ABI. All charts were reviewed by two independent reviewers who extracted information pertaining to the individual's history of substance abuse, whether substances were consumed at the time of injury, if recommendations were given to abstain and if the patient was offered addiction services/counseling.

Results: A total of 41 patients (33 males, 8 females) were found to have a history of drug (n=4) or alcohol (n=27) abuse, or both (n=10). Of those with a history of substance abuse, 15 patients had alcohol (n=12; range 9.2-78.8mmol/L) or drugs (n=2) in their blood, or both (n=1) at the time of injury. While all patients were recommended to abstain from consuming alcohol or drugs, only 15 out of 41 individuals with prior substance abuse were offered counseling services for their addiction; 10 individuals declined services and 5 were committed to contacting these agencies.

Discussion: Enrollment in drug and alcohol rehabilitation programs should be carefully considered for individuals post ABI; these programs may not be designed to specifically address the physical, cognitive and behavioural sequelae that often accompany the recovery process. Regardless, health care professionals should continue to offer these addiction services.

Oral Presentation

Presenter: Meghan Plotnik and Kate Mittermaier
Also Presented Poster Presentation

An aromatherapy study: effects of lavender on blood pressure and heart rate during mental stress.

Mittermaier, Katherine. Molley, Scott. Noushi, Niousshah. Paine, Paula. Plotnick Meghan, Rosenberg, Henri. Sun, Jonathon. Vitali, Sarah

Lavandula angustifolia, commonly known as English Lavender is marketed as a tool to create a relaxing environment and reduce stress. Increased heart rate (HR) and blood pressure (BP) are indicators of stress, and changes in these parameters are a useful measure of Lavender's affects. The purpose of the study on aromatherapy exposure during stressful situations was to analyze the efficacy of aromatherapy on reducing physiological signs of stress. It was hypothesized that lavender would decrease heart rate (HR) and blood pressure (BP) elevation in human subjects during a mentally stressful situation. Seventy-eight light to moderately active, non-smoking females aged 17-25 with a Body Mass Index (BMI) between 18.5-24.9 were recruited. Participants entered either an experimental 3% *Lavandula angustifolia* scented environment or a control non-scented environment. In each condition, subjects endured an initial five minute relaxation period, a five minute stressful period, and a five minute recovery period. Stress was induced in participants by having them complete a mathematics test. BP and HR were taken after each five minute period using the OMRON@ HEM278 Automatic Blood Pressure Monitor and Cuff. The data was then further broken down for analysis based on whether the female was in the first half of her menstrual cycle, second half of her menstrual cycle, or taking birth control. The change in BP from relaxed to induced stress conditions was 10 mmHg for control conditions and 10 mmHg in experimental lavender conditions. Changes in BP for control and Lavender were not significantly different ($P=0.274$). The HR changes from relaxed to stressed periods for control environment was 8 bpm and in lavender environment was 9 bpm. Changes in HR for control and Lavender were not significantly different ($P=0.436$). The data obtained did not support the hypothesis that lavender decrease the elevation in BP and HR in a stressful situation in the female subjects. Those data suggest that Lavender aromatherapy may be attributed to psychological effects instead of a tangible physiological response.