

**The Medical Student  
Summer Research  
Program  
of the  
University of Cincinnati  
College of Medicine**

**Poster  
Abstracts**

**Friday, October 11, 2002**

## **Blood Genomics of Stroke**

Matthew Wylie, Yang Tang MD PhD, Frank Sharp MD.  
Department of Neurology

**Introduction:** All types of brain injury induce an inflammatory response involving elements of the bloodstream supplying the CNS. Characteristics of this response may be reflected in the genomic expression of inflammatory mediators found in peripheral blood. The hypothesis for our study is that the genomic expression pattern observed in whole blood can act as “genomic fingerprint” identifying the injury that precipitated it. Using microarray technology we investigated genomic expression of rat whole blood following hypoxia, ischemia, seizure, and hypoglycemia compared to untouched and sham-surgery controls. We also examined whole blood expression in healthy controls and human patients following ischemic stroke and transient ischemic attack (TIA).

**Methods:** Adult rats were subjected to insult 24 hours prior to sacrifice, when brain and whole blood are harvested. Brain tissue was assayed for extent of neuronal injury and RNA was isolated from whole blood or leukocytes and applied to Affymetrix U34A microarrays. For the human study, patients were recruited from University Hospital and, if inclusion and exclusion criteria were met, a 20ml blood sample was drawn by peripheral venipuncture. Total RNA was isolated and applied to Affymetrix human U-133 Genechips. Data from both animal and human studies are processed for significance using Affymetrix Genechip software, Genespring software, and Significance Analysis for Microarray (SAM)

**Results:** Different genomic expression patterns were observed in rat whole blood following the different modes of insult, with expression of batteries of genes either increasing or decreasing in each case compared to each other, untouched rats, and sham-surgery rats. Furthermore, a unique expression profile was correlated with the presence of neuronal injury. Individual genes in this profile, such as VMAT-2, demonstrated a form of dose-response, with greater expression correlated with more severe injury. Results from the human study are still being processed.

**Conclusions:** These data indicate that various modes of neurological insult produce differing inflammatory responses (depending on severity of neuronal injury amongst other factors), mediated and traceable by changes in expression pattern in circulating blood cells. Individual genes in this profile may be useful in predicting the presence and extent of neuronal death following an insult. This is of great clinical significance as it suggests a cheap, simple, non-invasive method for diagnosing the type and severity of neurological insult in a human patient.

## Creatine Treatment and Diagnosis of Patients With Creatine Transporter Deficiency

Anish Wadhwa<sup>1</sup>; Joseph F. Clark, Ph.D.<sup>2</sup>; Ton J. deGrauw, M.D.<sup>3</sup>; Amy Newmeyer, M.D.<sup>4</sup>; Kim Cecil, Ph.D.<sup>5</sup>

<sup>1</sup>College of Medicine, UC; <sup>2</sup>Dept. of Neurology, Vontz Center for Molecular Studies, UC; <sup>3</sup> Dept. of Neurology, CHMCC; <sup>4</sup>Division of Developmental Disabilities, CHMCC; <sup>5</sup>Clinical Imaging Center, CHMCC

Creatine deficiency syndromes, which are inborn errors in brain metabolism of creatine, present clinically with various neurological symptoms that include extrapyramidal movement disorders, developmental regression, behavioral problems and intractable epilepsy. These congenital brain diseases, discovered and characterized within the past decade, are sorted into two major categories. The first category consists of deficiencies of enzymes, specifically guanidinoacetate methyltransferase (GAMT) and arginine-glycine-amidino transferase (AGAT), which are vital to the endogenous synthesis of creatine. Patients with GAMT deficiencies, when treated with oral creatine, demonstrate improvement of clinical symptoms and biochemical abnormalities, yet complete normalization of the clinical condition is not observed (Schulze et al. 1997). Unlike in patients with GAMT deficiency, oral creatine supplementation nearly completely restores pre-treatment creatine levels, as well as significantly improves the existing developmental deficits, in patients with AGAT deficiencies (Items et al. 2001). The second category includes deficiencies in brain creatine transporter activity. Identified as a X-linked phenomena affecting male children (and oftentimes their female carriers as well), patients treated with oral creatine show negligible rates of creatine uptake and thus no clinical improvement or increased creatine/phosphocreatine signal when analyzed by magnetic resonance spectroscopy (MRS) (Cecil et al. 2001).

In this Summer Research Fellowship project, the goal was to develop an assay which could assess the lack of creatine transporter activity in patients with the creatine transporter defect. Existing data demonstrates that carriers and patients of the creatine transporter defect have altered levels of creatine in the blood, blood cells and urine when compared to normal, healthy controls. In order to quantify these differences, we sought to develop an assay that could assess the relative activity of the creatine transporter by comparing intracellular vs. extracellular RBC creatine concentrations. Our unique adaptation to the standard fluorometric assay used to measure total tissue creatine (Conn 1960) seems to allow the reliable quantification of this ratio in a set of controls, thus providing an index of creatine transporter activity. In the future, we will look to characterize the differences in creatine concentrations of blood, serum and urine samples taken from diagnosed patients and healthy volunteers. The potential elimination of the multiple modalities currently used to diagnose the condition (MRS, magnetic resonance imaging, Western blot, muscle biopsy), as well as the establishment of diagnostic controls (for expected [Cr] values) for pediatric patients, will ultimately ease the identification of the population with defect as well as the screening of those patients who demonstrate similar clinical symptoms. Along with the other ongoing projects in the laboratory and clinical settings, it is anticipated that the assay will help to demonstrate proof of principle that oral Cr supplementation will increase (and possibly normalize) brain Cr in the population carrying the transporter defect.

## Calpain Inhibition Decreases Ischemia-Reperfusion Induced Pulmonary Hypertension and Cardiopulmonary Dysfunction by Decreasing Endothelin-1 in Neonates

Jason H. Bell, PhD; Jodie Y. Duffy, PhD; Connie J. Wagner; Jeffrey M. Pearl, MD

*Department of Cardiothoracic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229 USA*

*University of Cincinnati College of Medicine, Cincinnati, OH 45267 USA*

**OBJECTIVE:** Cardiopulmonary bypass (CPB) in infants and children can result in cardiopulmonary dysfunction through ischemia-reperfusion injury. Inhibition of calpain, a cysteine protease, has been shown to inhibit reperfusion injury. The hypothesis is that calpain inhibition can alleviate cardiopulmonary dysfunction after reperfusion in neonates.

**METHODS:** Crossbred piglets (5-7 kg) were cooled on CPB to 18° C followed by deep hypothermic circulatory arrest (DHCA) for 120 min. Animals were re-warmed to 37° C on CPB, and maintained for 120 min. Six animals were administered calpain inhibitor (*carbobenzoxy-leucinyll-leucinyll-tyrosine-fluoromethyl ketone*; 1mg/kg, IV) 120 min. prior to CPB. Nine animals were administered saline as a control. Plasma endothelin-1 levels were measured by enzyme-linked immunosorbent assay.

**RESULTS:** Calpain inhibition prevented the increase in pulmonary vascular resistance (PVR) compared with controls ( $95.7 \pm 39.4$  vs.  $325.3 \pm 83.6$  dyne•s/cm<sup>5</sup>, respectively;  $p < 0.06$ ). This decrease was associated with reduced plasma endothelin-1 levels ( $4.91 \pm 1.72$  after calpain inhibition vs.  $10.66 \pm 6.21$  pg/mL in controls,  $p < 0.05$ ). Pulmonary function 120 min. after reperfusion was improved with calpain inhibition compared with controls; PO<sub>2</sub>/FiO<sub>2</sub> ratio ( $507.2 \pm 46.5$  vs.  $344.7 \pm 140.5$ , respectively;  $p < 0.05$ ) and alveolar-arterial gradient ( $40.0 \pm 17.2$  vs.  $128.1 \pm 85.2$  mm Hg, respectively;  $p < 0.05$ ). In addition, oxygen delivery was increased with calpain inhibition compared to controls ( $759 \pm 171$  vs.  $277 \pm 46$  mL/min., respectively;  $p < 0.001$ ).

**CONCLUSIONS:** Calpain inhibition resulted in decreased endothelin-1 and an associated reduction in pulmonary vascular resistance. Improved gas exchange and cardiopulmonary function suggests that calpain inhibition may be advantageous in alleviating post-operative cardiopulmonary dysfunction commonly associated with neonatal cardiopulmonary bypass.

## Altered Metabolism of Postprandial Lipoproteins Leads to Diet Induced Obesity in Carboxyl Ester Lipase (CEL) Knockout Mice

Collin M. Burkart and Philip N. Howles, Ph.D.

Department of Pathology and Laboratory Medicine, University of Cincinnati, Cincinnati, OH

**Introduction:** Carboxyl Ester Lipase (CEL) is a broad spectrum lipase found in mostly in pancreatic juices, with a small amount found systemically. The CEL knockout and Control mice have been shown to absorb triglyceride and cholesterol at the same rate, but evidence has shown that the chylomicrons that these animals are secreting have different properties, with those of the CEL knockouts being smaller than Controls. There is also evidence which shows that CEL knockout mice gain more weight and have increased serum triglycerides and cholesterol than Controls on equal diets.

**Hypothesis:** The smaller particles that are secreted by the intestinal epithelial cells of CEL knockout mice are cleared more slowly by the liver as compared to the larger particles secreted by the control animals. As a result more dietary triglycerides are delivered to the peripheral tissues.

**Methods:** Lymph was collected from wild-type mice, CEL knockout mice and rats via a lymph fistula, during intraduodenal infusion of a lipid emulsion containing [<sup>3</sup>H]triglyceride, [<sup>14</sup>C]cholesterol. In the first experiment, the lymph collected from the wild-type and CEL knockout mice was injected into the jugular vein of Control mice. In the second experiment, the lymph collected from the rats was injected into the jugular vein of CEL knockout and Control mice. Blood was sampled from the tail vein of the injected mice. The animals were sacrificed various organs, fat pads and tissues were collected. Scintillation spectrometry was used to determine the amount of label recovered in the serum and tissue samples.

**Results:** In the first experiment, the rate at which the radiolabeled lipids were cleared from the serum was statistically identical. The second experiment, where rat lymph was injected into control and knockout mice, showed that the rate at which the [<sup>14</sup>C]cholesterol was cleared from the serum was the same, with high variability in the animals. However, the clearance of [<sup>3</sup>H]triglyceride in the second experiment was significantly different between the control and knockout mice. With the knockout mice clearing the [<sup>3</sup>H]triglyceride nearly twice as quickly.

Overall, the difference in distribution of percent recovered [<sup>3</sup>H]triglyceride and percent recovered [<sup>14</sup>C]cholesterol in nearly all of tissues collected was not significant between the mice injected with the wild-type and knockout lymph in the first experiment or between the control and knockout mice injected with rat lymph in the second experiment. However, for the first experiment there is a significant difference ( $p < 0.05$ ) in the percentage of recovered [<sup>3</sup>H]triglyceride in the lung and the heart with the mice injected with knockout lymph having a higher percentage in these

## Clinical Brain MRI prescriptions in Talairach space: Technologist and Computer Driven Methods

Kenneth L. Weiss, MD<sup>(1,2)</sup>, Hai Pan, MS<sup>(2)</sup>, Judd Storrs, BS<sup>(2)</sup>, William Strub, MD<sup>(1)</sup>, Jane L. Weiss, BSN<sup>(2)</sup>, Li Jia, MS<sup>(2)</sup>, O. Petter Eldevik, MD, PhD<sup>(3)</sup>

**BACKGROUND AND PURPOSE:** Variability in patient head positioning may yield significant inter-study image variance in the clinical setting. We describe and test 3-step technologist and computer-automated algorithms designed to image the brain in a standard reference system and reduce variance.

**METHODS:** Triple oblique axial images obtained parallel to the Talairach AC-PC plane were reviewed in a prospective analysis of 126 consecutive patients. Requisite roll, yaw, and pitch correction as determined independently and subsequently by consensus by three coauthors were compared to the technologists' actual graphic prescriptions and those generated by a novel computer algorithm. Automated pitch determinations generated with SPM99 were also compared.

**RESULTS:** Requisite pitch correction in degrees (15.2 +/- 10.2) far exceeded that for roll (-0.6 +/- 3.7) and yaw (-0.9 +/- 4.7) in terms of magnitude, and variance ( $p < 0.001$ ). Technologist/computer generated prescriptions significantly reduced interpatient image variance with regards to roll (3.4/3.9 vs. 13.5), yaw (0.6/2.5 vs. 22.3) and pitch (28.6/18.5 –novel and 59.3 – SPM vs. 104). The novel computer algorithm performed less well than the technologist in yaw prescription, and equivalently in roll and pitch prescriptions. Talairach prescriptions better approximated standard CT cantho-meatal angulations (9 vs. 24 degrees) and provided more efficient brain coverage than routine axial imaging.

**CONCLUSION:** Direct roll, yaw, and Talairach AC-PC pitch corrected brain MR prescriptions can be readily achieved by trained technologists or automated computer algorithm significantly reducing intersubject variance, better approximating standard CT angulation, and yielding more efficient brain coverage than routine clinical axial imaging.

Student: Shaun M. Stickley  
Preceptor: Karl Matlin, PhD  
Department of Surgery, Epithelial Pathobiology Research Group

**Title:**  
Deposition of Laminin-5 During Epithelial Cell Polarization

**Abstract:**  
Epithelial cells are polarized along an axis running from the free apical surface to the base of the cells. This polarization affects not only the organization of the cytoskeleton, positioning of organelles, and the location of intercellular junctions, but also resulting in the creation of plasma membrane domains with distinct protein and lipid compositions. Epithelial polarity is crucial for all differentiated functions of epithelia, including secretion and ion transport. Disruption of the normal epithelial polarity is a common feature in many pathological processes. Epithelial cells polarize in response to cues from the substratum and cell-cell contacts. However, little is known about which cell-substratum interactions provide key polarization signals. When Madin-Darby canine kidney (MDCK) cells are plated at high density, they polarize within 18 hours. During this period, analysis of proteins secreted and deposited in the substratum by metabolic labeling and SDS-gel electrophoresis indicated that a number of high molecular weight polypeptides are synthesized, and that the overall composition of the matrix changes over time. When extracts of this endogenous matrix were probed by immunoblotting with an antibody against the laminin  $\beta 3$  chain, a component of laminin-5 ( $\alpha 3\beta 3\gamma 2$ ), then deposition of  $\beta 3$  was noted 3-6 hours after initial plating. When longer culture times were examined, it appeared that the concentration of  $\beta 3$  began to decline after 24 hours and was almost undetectable 7 days after initial plating. Extracellular matrix extracts were further probed with additional antibodies indicating the presence of the laminin-5  $\alpha 3$  chain. RT-PCR analysis of RNA preparations from MDCK cells indicated that  $\beta 3$  transcripts were detectable, confirming the identification of the  $\beta 3$  by immunoblotting. These observations are significant because they correlate with parallel *in vivo* studies suggesting that laminin-5 is synthesized in the postischemic rat kidney during epithelial regeneration, repolarization, and redifferentiation, and imply that cell interaction with laminin-5 may provide a key signal for cell polarization.

tissues. In this same experiment, there is also a significant difference ( $p < 0.05$ ) in the percentage of recovered [ $^{14}\text{C}$ ]cholesterol in the brown fat with the mice injected with knockout lymph having a higher percentage. The trend of difference in distribution between the tissues did show that either the Control mice injected with knockout lymph or the knockout mice injected with rat lymph had a higher percentage of recovered [ $^3\text{H}$ ]triglyceride in the tissues collected and less in the liver and these same mice had a higher percentage of recovered [ $^{14}\text{C}$ ]cholesterol in the tissues collected other than the liver.

**Conclusions and Significance:** The clearance results from the two experiments suggest that the particle specific effects of the enzyme do not affect the clearance by the liver. This is shown in the first experiment where the clearance rates of the control mice injected with knockout and wild-type mice are the same. However, the results from the clearance rate in the second experiment suggest that the enzyme does have significant systemic effects on clearance by the liver. The knockout mice clear the control rat lymph nearly twice as fast as the wild-type mice.

The tissues distribution results from the two experiments demonstrate that there is a trend which is in agreement with the hypothesis indicating that the liver is clearing less of the lipids while the peripheral tissues are clearing more of these lipids. The difference in this distribution is not shown to be significant in nearly all of the tissues and a larger sample size is needed to see if the knockout mice are in fact collecting more of the labeled lipid in tissues other than the liver.

This data could indicate that the knockout mice have somehow adapted to clear particles more quickly because they must clear smaller particles than the control on a routine basis. One possibility is that there could be an increased expression of receptors for these particles in the peripheral tissues. Therefore, when the larger particles are injected they are cleared more quickly by the peripheral tissues. These results are significant because it indicates that CEL does have an effect on clearance and possibly tissue distribution of lipids and this effect seems to be carried out systemically.

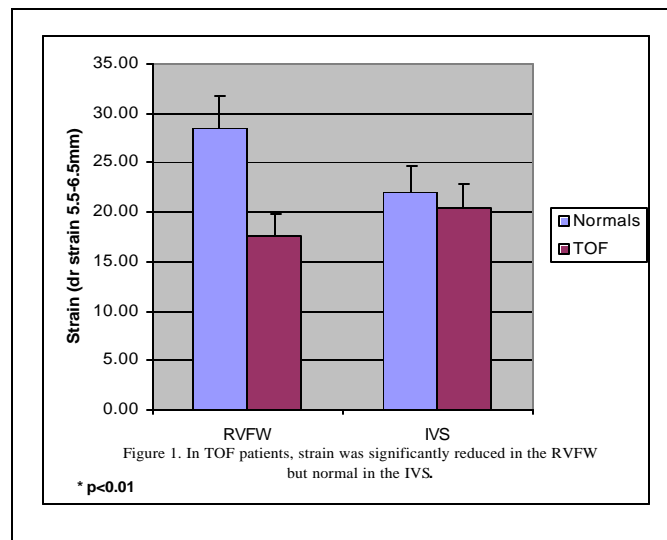
## Induction of resistin-like molecule beta (RELMb) by respiratory allergen, IL-4, IL-13, and STAT6 in experimental asthma.

C.W. DeBrosse, N. Zimmermann, N. E. King, S.M. Pope, P.C. Fulkerson, A. Mishra, and M.E. Rothenberg. Children's Hospital Medical Center, Cincinnati, OH 45229.

**Rationale:** Prompted by the current rising incidence and severity of asthma despite intense ongoing research efforts, we utilized an unbiased, empiric approach involving transcript profiling of asthmatic lung RNA. **Methods:** Lung RNA from asthmatic and control mice were subjected to transcript profile analysis using Affymetrix microarrays that contained the largest commercially available collection of murine genes. The data obtained from these experiments demonstrated a strong induction of RELM $\beta$  in the lungs of asthmatic mice. RELM $\beta$  is a member of the resistin family of proteins, a structurally related group of cytokines that have been associated with resistance to insulin (obesity). In order to more fully characterize the activity of this cytokine during allergen induced asthma, northern blot analysis of wild type, allergen induced and cytokine over expressing gene targeted mice was performed. **Results:** Employing asthma models induced by different allergens and protocols, we found strong induction of the mRNA for RELM $\beta$ . RELM $\beta$  mRNA was not detectable in control lungs, but was markedly increased following the development of OVA (ovalbumin) and *Aspergillus fumigatus* antigen-induced experimental asthma. A time and dose dependent expression of RELM $\beta$  in OVA challenged mice was demonstrated. RELM $\beta$  was also induced in the lungs of IL-4 transgenic mice and by intratracheal treatment with recombinant IL-13. Using mice deficient in signal-transducer-and-activator-of-transcription (STAT)6, allergen and IL-4/IL-13-induced RELM $\beta$  expression was demonstrated to be dependent upon this transcription factor. In contrast, IL-5 deficient mice had normal induction of RELM $\beta$  compared with wild type mice.

**Conclusion:** RELM $\beta$  is induced by allergen, IL-4, and IL-13 by a STAT-6 dependent mechanism in the asthmatic lung. These results provide a mechanistic link between the pathogenesis of insulin resistance (obesity) and asthma.

and SR correlated moderately with reduced RVEF ( $r = -0.49$ ,  $p = 0.08$ ) and greater PR ( $r = 0.48$ ,  $p = 0.08$ ). In post-operative TOFs, strain and SR were reduced primarily in the RVFW, whereas these indices were similar in the IVS (see Figure 1).



**CONCLUSION:** In the post-operative tetralogy of Fallot population, systolic and diastolic right ventricular myocardial strain and SR were impaired in the RVFW but preserved in the IVS. We speculate that IVS myocardial function is preserved as a compensatory mechanism for impaired RVFW function.

## **Right ventricular myocardial strain and strain rate in post-operative tetralogy of Fallot patients: the elusive goal of evaluating right ventricular function**

Solarz DE, Glascock BJ, Jones FD, Khoury PR, Witt SA, Kimball TR

Non-Invasive Cardiac Imaging and Hemodynamic Research Laboratory, Division of Cardiology, Cincinnati Children's Hospital Medical Center

**BACKGROUND:** Clinical evaluation of RV function is critical in post-operative tetralogy of Fallot (TOF) patients but technically problematic. New indices such as strain and strain rate (SR) may be promising when applied to the RV because they are free of geometrical assumptions.

**PURPOSE:** To evaluate RV strain and SR in post-operative TOF patients versus normals and to determine the functional correlates of strain and SR in post-operative tetralogy of Fallot patients.

**METHODS:** All post-operative (>1 year) tetralogy of Fallot patients were eligible. Echo was performed in the apical four-chamber view with in the quiet, resting state. Strain and SR indices were measured along the lateral free wall of the RV (RVFW) and the interventricular septum (IVS) using a semi-automatic frame-by-frame tissue tracking (dr strain 5.5-6.5 mm). Pulmonary regurgitation (PR), pulmonary stenosis (PS), QRS duration, and RV pressure, thickness, and dimension were also measured. These indices were compared to normal controls. Intra- and inter-observer variation were calculated.

**RESULTS:** There were 15 post-operative ( $6\pm 2.8$  years) tetralogy of Fallot ( $7\pm 4$  years of age) patients and 25 normal patients ( $10\pm 5$  years of age). There was no significant difference between the TOF and normal population for age ( $p=0.51$ ). Intra- and inter-observer variability were 89% and 80%, respectively. SR and strain in the RVFW were significantly reduced in TOFs relative to normals ( $p=0.01$  and  $<0.03$ , respectively). There were no significant differences between the TOF and normal population for SR and strain in the IVS (Figure 1). In the RVFW, strain and diastolic SR correlated with greater PS ( $r=0.7$ ;  $p<0.05$ ), RV pressure ( $r=0.66$ ,  $p=0.05$ ) and RVEF ( $r=0.64$ ,  $p<0.01$ ). In the IVS, systolic strain

## **Thrittene (S-13) Expression in the Gastric Mucosa**

Timothy Duerler

**Introduction:** Recently a peptide has been isolated from the mammalian GI tract that has considerable homology to the N-terminus of S-28 (1-2). This peptide, S-28 [1-13] (S-13), or thrittene, has not been previously described but is produced in endocrine cells in the stomach, intestine and pancreatic islets similar to the somatostatins (1-2). However, S-13 is also synthesized in GI neurons that do not contain somatostatin immunoreactivity. In addition, S-13 is produced in the GI tissues of mice with a targeted gene deletion of the prosomatostatin gene (1). These data indicate that S-13 is produced independently from prosomatostatin, possibly from a novel gene.

**Rationale and/or Hypothesis:** It is important to determine thrittene's physiologic role. One of the ways in which this can be done is through examination of the tissue distribution. The stomach provides an important organ system because the gastric D endocrine cells are known to produce large amounts of somatostatin and it has previously been demonstrated that S-13 is produced in the gastric endocrine cells in prosomatostatin knockout mice. Since it has been shown that S-13 and the prosomatostatin active metabolites are produced independently we hypothesized that the two peptides may be produced in entirely different gastric endocrine cells.

**Methods:** Immunofluorocytochemistry was used in order to test the hypothesis that S-13 and the products of prosomatostatin (S-14 and S-28) are produced and found in different cells. A dual-labeling experiment was set up using as primary antibodies MS12, a monoclonal mouse antibody that reacts with a C-terminal epitope on S-14 and S-28, but does not recognize S-13, and a polyclonal rabbit antisera that was raised against S-13. Fluorescently-labeled goat anti-mouse Cy3 (fluoresces red) and goat anti-rabbit AlexaFluor (fluoresces green) were added as secondary antibodies and the slides were analyzed using fluorescent microscopy.

**Results:** An S-13+ endocrine cell fluoresced green, an S-14/S-28+ cell fluoresced red, and using a dual filter co-localization of the two peptides showed as yellow. Images of the gastric mucosa were captured on a digital camera and analyzed. By visual examination, the majority of cells fluoresced yellow, with a small number showing only green-staining. A further quantitative analysis was done by capturing successive images of the gastric mucosa until 50 cells were counted. Quantitation was done by counting every cell as having co-localization (yellow) or S-13+ only (green with the absence of red). ~90% of the staining was co-localized and 10% of the cells expressed S-13 only without the somatostatins.

**Conclusions & Significance:** Whenever a novel peptide is isolated it is important to understand its physiologic role, especially if it shares a sequence homology with part of a significant known peptide hormone such as somatostatin. From this experiment it has been shown that the majority of S-13 produced in the gastric mucosa does indeed come from the same endocrine cells that express prosomatostatin and synthesize S-14 and S-28. However, a small population of gastric mucosal cells exist that produce S-13 only, and do not contain the somatostatins. This finding provides further support for the independence of S-13 from prosomatostatin and may help to elucidate further functions of S-13 and its effects.

## Evidence For Enhanced Ischemic Preconditioning Through Combination dPKC-Inhibition and ePKC-Activation

K. Chad Hilty§, Faisal Syed, Yehia Marreez, Amy Odley, Harvey S. Hahn\*, and Gerald W. Dorn II

University of Cincinnati College of Medicine, Department of Internal Medicine, Division of Cardiology, Cincinnati, OH 45267

§ research fellow, \* project mentor

### Introduction

Ischemic pre-conditioning, which is defined as the resistance of heart tissue to prolonged ischemia after an initial period of transient ischemia, is the second most significant form of myocardial protection, after reperfusion therapy known to date.<sup>1,2</sup> Six protein kinase C (PKC) enzymes have been shown to translocate to the membrane upon activation in cardiac myocytes, and among these dPKC and ePKC have been linked to ischemia preconditioning.<sup>3</sup> Earlier work in the Dorn lab has demonstrated that the use of a rationally-designed ePKC agonist, pseudo-eRACK (?eRACK), can induce ePKC activation, translocation, and sustained ischemic preconditioning.<sup>4</sup> Activation of dPKC, on the other hand, has been shown to increase ischemic damage in the cardiac myocyte.<sup>5</sup> Recent work in the lab has focused on studying the effect of inhibiting dPKC activation and translocation with a peptide inhibitor, dV1. Inhibition of dPKC with dV1 is indeed cardioprotective and effective at ischemic preconditioning.<sup>6</sup> Additionally, inhibition of dPKC or activation of ePKC alone both confer a reduction in ischemic damage greater than 50%.<sup>5</sup>

### Hypothesis

Simultaneous activation of e PKC and inhibition of dPKC is more cardioprotective than either treatment alone.

## RESULTS

Following the established lag time for TNF- $\alpha$  stimulation of 40min,<sup>3</sup> PMNs adherent to MV plates showed an average increase of 1000% in hydrogen peroxide production in response to TNF- $\alpha$  compared to the buffer control. Those adherent to FN plates showed an 80% increase, while those adherent to FBG showed a 30% increase.

## CONCLUSIONS

The mechanism of linkage for PMNs to the vascular wall before their movement into the interstitial space is mediated by  $\beta$ 1 and  $\beta$ 2 integrins among other factors. Binding by  $\beta$ 1 and  $\beta$ 2 integrins can be reproduced separately *in vitro* on FN and FBG plates respectively. Our experiments suggest that  $\beta$ 1 integrin binding is sufficient to stimulate hydrogen peroxide production by neutrophils (Fig. 1) but that this binding does not upregulate the cell to further stimulation by cytokines such as TNF- $\alpha$  when compared to the MV response (Fig. 2). PMNs adherent via  $\beta$ 2 integrins on FBG neither showed activation on binding alone (Fig.1) nor significantly greater activation on TNF- $\alpha$  stimulation than those on MV plates (Fig. 2). This data suggests that microvascular endothelial cells serve as a platform for upregulating neutrophils for further responses to cytokines such as TNF- $\alpha$ , possibly by the synergistic effect of  $\beta$ 1 and  $\beta$ 2 integrins and/or other receptor classes. A better understanding of the mechanisms that regulate neutrophil responses to inflammation will reduce the morbidity and mortality associated with major injury and trauma.

<sup>1</sup> Kloner, R.A., Bollix, R., Marban, E., Reinlib, L., and Braunwald, E. *Circulation*. 1998; 97: 1848 -1867.

<sup>2</sup> Murray, C.E., Jennings, R.B., & Reimer, K.A. *Circulation*. 1986; 74: 1124 -1136.

<sup>3</sup> Gray, M.O., Karliner, J.S., & Mochley-Rosen, D. *Journal of Biological Chemistry*. 1997: 272: 30945-30951.

<sup>4</sup> Dorn, G.W. II, et.al. *PNAS*. 1999; 96: 12798-12803.

<sup>5</sup> Chen, L., Hahn, H., Wu, G., Chen, C.H., Liron, T., Schechtman, D., Cavallaro, G., Banci, L., Guo, Y., Bolli, R., Dorn, G.W., and Mochly-Rosen, D. *PNAS*. 2001; 98: 11114-11119.

<sup>6</sup> Hahn, H.S., Yussman, M.G., Toyokawa, T., Marreez, Y., Barrett, T.J., Hilty, K.C., Osinska, H., Robbins, J., and Dorn, G.W.

*Circulation Research*. 2002. In Press.

# Human Neutrophil Peroxide Production in Response to Tumor Necrosis Factor- $\alpha$ (TNF- $\alpha$ ) is Enhanced by Adherence to a Microvascular Endothelial Cell Monolayer *in vitro*

Keith R. Ridel, Cindy M. Cave, Joseph S. Solomkin, MD

## INTRODUCTION

Human polymorphonuclear leukocytes (PMN) or neutrophils must pass from the vascular space in order to assert their proinflammatory response to major injury and trauma. Transcytosis is mediated by both direct neutrophil-endothelial interaction and cytokine gradients presented within the vascular lumen, resulting in PMN cytoskeletal rearrangements and hydrogen peroxide production<sup>7</sup>. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) has been shown to be released in response to endotoxic shock as well as induce rolling, adhesion, diapedesis, and congregation of PMNs at a site of injury<sup>2</sup>. Therefore, a functional assay that measures hydrogen peroxide production of PMNs adherent, *in vitro*, to fibronectin (FN) via  $\beta$ 1 integrins, fibrinogen (FBG) via  $\beta$ 2 integrins, or a microvascular endothelial cell monolayer (MV) would give an indication of the relative abilities of each to serve as a platform for PMN stimulation by TNF- $\alpha$ .

## HYPOTHESIS

PMNs adherent to a microvascular endothelial cell monolayer show markedly increased hydrogen peroxide production in response to TNF- $\alpha$  stimulation relative to those on fibronectin or fibrinogen alone.

## METHODS

Neutrophils were isolated from the whole blood of healthy volunteers by dextran sedimentation and Histopaque 1077 centrifugation followed by removal of red blood cells via hypotonic lysis. Neutrophils were then incubated at 37°C for 10min in 96 well plates coated with either FN, FBG, or MV and subsequently stimulated with buffer (KRPB) or 10ng/ml TNF- $\alpha$ . Hydrogen peroxide production was measured at 10min intervals for 80min as the decrease in fluorescence due to the oxidation of scopoletin.

<sup>7</sup> Miyabayashi, M., K. Yasui. Regulation of neutrophil O<sub>2</sub><sup>-</sup> production by neutrophil-endothelial interaction via CD11b: its modulation by tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and lipopolysaccharide (LPS). *Int J Hematology* 1996; 65: 49-59.

<sup>2</sup> Lawrence, MB, TA Springer. Neutrophils roll on E-selectin. *J Immunol* 1993; 11: 6338-6346.

<sup>3</sup> Williams, MA, et. Al. Chemokine Regulation of Neutrophil Function in Surgical Inflammation. *Arch Surg* 1999; 134: 1360-1366.

## Methods

Experimental mice were anesthetized with avertin i.p. (per protocol) and their hearts were rapidly removed to reduce ischemia and cannulated via the aorta in a Krebs-Henseleit buffer on a Langendorff *ex vivo* perfusion apparatus. Left ventricular pressure and real time derivative (dP/dt) was measured via a catheter in the apex of the ventricle. The heart was perfused for 20 minutes to allow equilibration. Simulated ischemia was induced by interruption of the perfusate for 40 minutes, followed by 30 minutes of reperfusion. Measurements were made at 1 minute intervals throughout the reperfusion period. Cardiac damage was assessed using the diagnostic marker creatine kinase (CK) via an *in vitro* assay kit (Sigma) performed on the post-ischemic perfusate. Samples were rapidly frozen to preserve CK activity and allowed to thaw completely at room temperature before being assayed.

## Results

Mice that simultaneously expressed the dV1 and  $\gamma$ eRACK peptides had a more rapid and greater return to baseline hemodynamic function as measured by left ventricular pressure and real-time derivative (~2900 mmHg/sec) as compared to  $\gamma$ eRACK (~2600 mmHg/sec) and NTG (~2000 mmHg/sec) alone. Creatine kinase release values were comparable in crossed dV1 and  $\gamma$ eRACK mice (168.50  $\pm$  38.65 IU) to those in  $\gamma$ eRACK (115.00  $\pm$  33.32) mice and non-transgenic (575.00  $\pm$  298.00) mice.

## Conclusions

The data suggest that simultaneous expression of dV1 and  $\gamma$ eRACK peptides in mice has a greater cardioprotective effect than expression of either peptide alone. CK activity of the crossed mice do not show a significant decrease in ischemic injury over either peptide alone. The reduction in CK activity is significant when compared to non-transgenic mice and comparable to either of the CK levels in the mice who expressed the proteins separately. More work needs to be done to further define the roles of dPKC and ePKC in ischemic pre-conditioning. Future work will also aim at exploiting the potential therapeutic applications of dPKC inhibitor and ePKC activator proteins in inducing ischemic pre-conditioning and mitigating cardioprotection.

# **Serum After Small Bowel Resection: Effects on Apoptosis in Intestinal Epithelial Cells**

Blank Page

Gregory S. Huang, Russell Juno, M.D., Christopher R. Erwin, Ph.D., Brad W. Warner, M.D.

Division of Pediatric Surgery, Department of Surgery  
Children's Hospital Medical Center

## **Introduction**

Following massive small bowel resection (SBR), the remnant intestine compensates for the loss of mucosal surface area by undergoing intestinal adaptation. This response is characterized by increased rates of both enterocyte proliferation and apoptosis. The mechanisms and/or mediators of these responses are not presently understood. Prior work in the Warner laboratory identified significant changes in the expression of specific members of the bcl-2 gene family (bax and bcl-w) after SBR, which may be important in the regulation of apoptosis. Further, this laboratory has demonstrated that serum, when harvested from animals after SBR, induces proliferation of intestinal epithelial cells *in vitro*.

## **Rational**

This project tested the hypothesis that like proliferation, enterocyte apoptosis is increased by SBR serum *in vitro*. Further, since alterations in bax and bcl-w expression have been identified after SBR *in vivo*, we sought to determine whether the expression of these genes are affected by SBR serum *in vitro*.

## **Methods**

Rats underwent a 75% mid SBR or sham operation and serum was harvested 24 hours later. The Sham and SBR serum was added to a prototypical rat intestinal epithelial cell line (RIEC-6) and apoptosis was recorded by immunostaining for DNA strand breaks. The expression of bcl-2 family members (bax and bcl-w) protein was determined by Western blotting.

## **Results**

Apoptosis was not induced by addition of SBR serum at early, mid, or late time points. Further, over a wide range of time points (1 hour to 5 days), the effect of SBR serum on the expression of bax or bcl-w protein was variable and inconsistent, and did not coincide with histologic evidence for apoptosis

## **Conclusions & Significance**

Unlike proliferation, the induction of enterocyte apoptosis after massive SBR is likely regulated by factor(s) that do not circulate in the serum. These results support the concept that proliferation and apoptosis are independent pathophysiologic responses to massive SBR, and these are likely governed by different mechanisms.

## **Hispanic mothers' comfort about domestic violence screening compared with other routine screening questions**

Student: Brooks Keeshin

Advisor: Therese Zink, MD, MPH

**Introduction** One fourth of all women experience domestic violence at some point during their lives. Preliminary studies show that Latina women in the United States are less likely to report abuse, have a greater tolerance of severe abuse and are more likely to stay in an abusive relationship than their Anglo counterparts. Many organizations, including the American Medical Association and the American Academy of Pediatrics recommend that physicians screen routinely without children or partner present. Because mothers accompany their children 85 percent of the time to pediatric visits, screening the mother alone is logistically difficult in a busy practice. Furthermore, many doctors report that they don't want to offend the patient by asking about domestic violence (DV). Research has shown that it may be acceptable to screen mothers for DV using general questions in front of their children.

**Rational/Hypothesis** The purpose of our study is to evaluate the acceptability of general DV screening questions for women in Spanish compared with other routine sensitive screening questions when their children (3-12 yrs.) are with them in the primary care setting.

**Methods** 46 Spanish-speaking mothers with children ages 3-12 were interviewed at community centers, churches and in their homes without children present. The survey composed of validated questions for drug/alcohol use, sexual activity, depression and general DV questions. The mother was asked to indicate her comfort level in answering each question alone and if her child were present using a 5-point Likert scale. The participants were then asked to rank the five general DV questions from most comfortable to answer in front of their children to the least comfortable in front of their children.

**Results** Latina mothers were most comfortable answering sensitive issue screening questions alone. They indicated greater comfort with the DV screening questions: Do you feel safe in your current relationship and how is your partner treating you and the kids, than with questions about sex, depression and with the DV questions that talked about tension in the relationship and arguments, significance ( $\leq 0.005$ ). When subjects were examined by income, higher income ( $>20,000$  per year, N=11) reported no significant differences in any of the DV questions whereas those in the lower income cohort ( $<20,000$ , N=35) reported significant differences with the two DV questions (tension and arguments). When asked to rank questions based on most comfort in front of the children, the questions with "safe" and "treat" were preferred.

**Conclusions and Significance** Helping doctors understand that Latina mothers are more comfortable about general screening questions for DV than they are about screening for sexual activity and depression in front of their children may make physicians more willing to do routine DV screening. However, Latina mothers are uncomfortable with DV screening questions that include "tension" and "argument." More research is needed to appreciate differences concerning DV screening among Latina women living in the United States.

Geoffrey Rechenberg

Preceptors: Dr. Eric Gruenstein, Ph.D.  
Michelle Sellers, B.S.

**Title:** “Decreased frequency of synchronized cytoplasmic calcium oscillations in an in vitro network of cortical neurons following chronic, but not acute, lithium treatment”

**Introduction:** Although lithium ( $\text{Li}^+$ ) has proven to be one of the most effective treatments for bipolar disorder over the past fifty years, its therapeutic mechanism of action is still unknown. It has been hypothesized that bipolar disorder may result from an imbalance in excitatory (glutamate) and inhibitory (GABA) neurotransmission. Interestingly, it was recently demonstrated that chronic, but not acute, lithium modulates glutamate activated signaling through the NMDA receptor in primary cultures of CNS neurons. Chronic lithium reduced NMDA receptor-mediated calcium influx by approximately 50%, thereby protecting neurons from glutamate induced excitotoxicity. NMDA receptors are also important mediators of another protective mechanism that neurons utilize to avoid potential toxic effects of calcium, i.e. signaling through cytoplasmic calcium oscillations rather than continuously elevated calcium levels. It is believed that these oscillatory calcium signals may carry information encoded in their amplitude and/or frequency which may result in specificity of gene expression. Since lithium reduces calcium influx through NMDA receptors, it may also have important effects on neuronal signaling through cytoplasmic calcium oscillations.

**Hypothesis:** It is hypothesized that lithium will decrease the amplitude and/or frequency of cytoplasmic calcium oscillations through its effect on NMDA receptors in an in vitro network of rat cortical neurons.

**Methods:** Dissociated cortical neurons from 17-day rat embryos form a synaptically connected network in vitro. After approximately 10 days, this network undergoes spontaneous synchronized calcium oscillations. Calcium-sensing fluorescent dyes, microphotometry and video imaging were used to detect changes in frequency and amplitude of intracellular calcium oscillations. Non-treated cultures were compared to those treated with 5 mM lithium for either 1 day, 3 days or 8 days.

**Results:** The effects of lithium on frequency and amplitude of calcium oscillations were determined for acute (1 day), intermediate (3 day) and chronic (8 day) treatments. Following the 8 day treatment, a significant decrease in frequency was observed when compared to the non-treated cells. Normalizing the non-treated group to 100%, 8 day lithium treatment decreased oscillation frequency to 49% ( $\pm 12$ ) of the non-treated group ( $100 \pm 24$ ). Furthermore, a scattergram revealed little overlap in oscillation frequency of 8 day lithium and non-treated cells. There were no significant differences in frequency for either the 1 or 3 day lithium treatment groups. Likewise, there were no significant differences observed in the amplitude of calcium oscillations following any of the lithium treatment periods.

**Conclusions and significance:** Consistent with our hypothesis, chronic, but not acute, lithium treatment resulted in a significant decrease in the frequency of cytoplasmic calcium oscillations in a network of cortical neurons. Since it has been shown that chronic lithium significantly reduces calcium influx through NMDA receptors, it may be somewhat surprising that there were no significant changes in the amplitude of calcium oscillations.

The therapeutic effects of lithium may not become apparent in bipolar patients for up to two weeks or more. This suggests that changes in gene expression may be important to lithium's therapeutic mechanism. Since intracellular calcium is an important mediator of both pre- and post-synaptic signal transmission as well as neuronal gene expression, it raises the interesting possibility that modulation of calcium signaling by lithium may be important to its therapeutic mechanism.

**Future Studies:** Since lithium is toxic at higher concentrations, dose-response experiments would identify optimal concentrations and determine therapeutic relevance. In addition, lithium effects on sensitivity of glutamate receptor subtypes (NMDA, AMPA) may further elucidate the effects of lithium on excitatory neurotrans

