SCA37

RAT CHROMOSOME 18 SUBSTITUTION-DEPENDANT DIFFERENCES IN CARDIAC FUNCTION ARE ASSOCIATED WITH ANESTHETIC PRECONDITIONING

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Introduction: Isoflurane produces a delayed preconditioning (APC) against myocardial ischemia and reperfusion injury.[1] The objective of this study was to compare cardioprotective effects afforded by APC in two genotypically distinct parental strains of rats (Dahl Salt Sensitive [SS] and Brown Norway [BN]) and in a consomic (chromosomal transfer) strain (SS-18BN). The latter was a strain available from a larger panel of consomics[2] in which single chromosome from the BN strain has been introgressed into an otherwise unchanged SS genetic background.[3]

Methods: APC trigger was achieved in vivo by 120 min exposure to isoflurane (1.0 minimum alveolar concentration) with an inspired oxygen concentration of 30% in five male adult (9-12 weeks-old) animals of each strain. Respective control groups were not subjected to above treatment. Twenty-four hours later hearts were isolated and subjected to 30 min of global, no-flow ischemia, and cardiac function was assessed during 120 min of reperfusion using the Langendorff model. Data are means ± SEM ($p < 0.05$; * vs. BN).

Results: Isoflurane significantly improved functional recovery in BN and SS-18BN strains (with rate-pressure product averaging 28,550 ± 2,505 and 27,620 ± 3,055, respectively) as compared with SS strain (17,555 ± 2,990*). There were no significant differences in cardiac function between three control groups.

Discussion: The major significance of these results is the demonstration of a significant and reproducible difference in cardioprotective effects afforded by APC between two inbred strains that can be changed by a single chromosomal substitution. This knowledge provides a basis for selectively studying genetic factors associated with mechanisms of delayed anesthetic preconditioning related to this chromosomal substitution.

References: 1) Anesthesiology 100: 525-31, 2004. 2) PhysGen. Parental and Cosomic Strain Profiles 2006. (http://pga.mcw.edu/pga/) 3) Acta Physiol Scand 181: 585-92, 2004. Acknowledgments: Supported in part by grant No. 0360042Z from the American Heart Association-Greater Midwest Affiliate and PO1GM066730 from the National Institutes of Health.

SCA38

NITROSATIVE STRESS AND SERCA2 ACTIVITY IN CARDIAC SURGICAL PATIENTS

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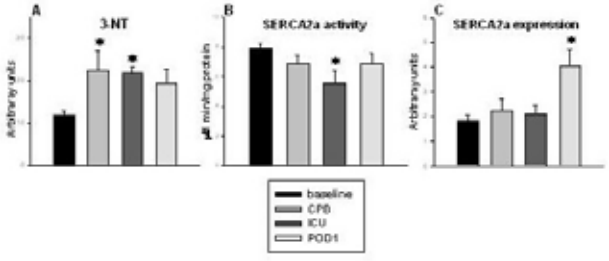
Cardiac surgery involving cardiopulmonary bypass (CPB) is associated with an acute, systemic oxidative stress. Under these conditions, superoxide and nitric oxide can interact in tissues to produce highly reactive peroxynitrite (ONOO-) that, in turn, transiently nitrosylates cysteine and permanently nitrates tyrosine residues within cellular proteins. Data indicate that the calcium cycling protein sarcoplasmic endoreticular calcium ATPase subtype 2 (SERCA2) is inhibited by chronic ONOO- exposure, a finding linked to contractile dysfunction in end-stage cardiomyopathy. SERCA2 is expressed as the 2a isoform in the heart and slow-twitch skeletal muscle and as the alternative-splicing product 2b in platelets and other tissues. Whether acute nitrosative stress in the clinical setting influences activity of either SERCA2 isoform is unknown. Based upon preliminary data indicating a marked sensitivity of SERCA2b to ONOO(IC50 in the low picomolar range) we hypothesized that platelets may represent an easily accessible model system for monitoring nitrosative changes in SERCA function during and after cardiac surgery with CPB. In order to test this hypothesis, platelet ONOO- generation and SERCA2b activity were determined in the perioperative setting.

Methods: Under an IRB-approved protocol, platelets harvested from 7 patients undergoing CABG with CPB and hypothermic cardioplegia were analyzed for the study. For each patient, blood samples were obtained prior to skin incision (baseline), during CPB but prior to aortic cross-clamping (CPB), upon arrival in the ICU (ICU), and on the first postoperative day (POD1). Nitrosative stress was expressed as total 3-nitrotyrosine (3-NT), a stable product of ONOO-, in platelet preparations. SERCA2b expression was assessed by Western blot while activity was determined by ATP hydrolysis assay. Data are presented as mean + SE and were analyzed with ANOVA for repeated measures with $p< 0.05$ considered significant.

Results. As shown in figure panel A, with CPB there was a rise in 3-NT that was associated with a subsequent decline in SERCA2b activity but no reduction in expression. Postoperatively, 3-NT remained modestly elevated but SERCA2b activity was not different than baseline, perhaps as a consequence of increased SERCA2b expression.

Conclusions. The data demonstrate that platelets are subjected to nitrosative stress during and after CPB that is associated with a reduction in SERCA2b activity. Although the data do not provide direct evidence of SERCA2b modification by ONOO-, given the lack of a concomitant decline in SERCA2b expression and the sensitivity of SERCA2b activity to picomolar concentrations of ONOO-, there is high probability that tyrosine nitration within the protein modulated the observed effects. These preliminary data suggest that CPB may induce nitrosative changes in SERCA2 isoforms, as well as other proteins, that have persistent functional implications.



ANESTHANALG 2007;104,SCA1-123

SCA39

ASSOCIATION OF THE 98G/T ELAM-1 POLYMORPHISM WITH INCREASED BLEEDING AND TRANSFUSION AFTER CARDIAC SURGERY

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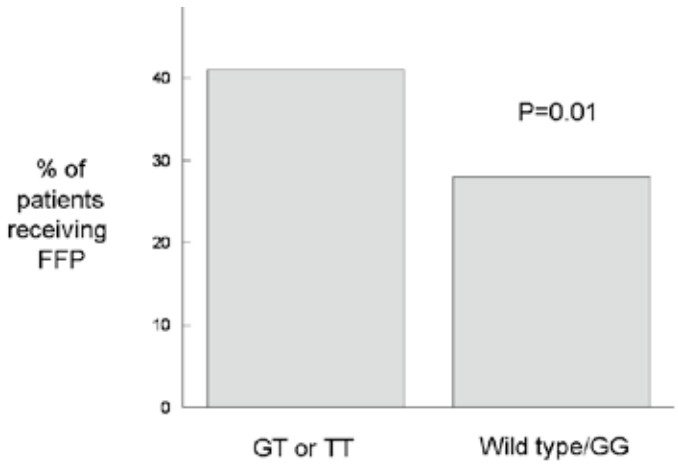
Background: Bleeding continues to be a major problem after cardiac surgery with inter-patient variability poorly explained by already known risk factors. A genetic basis for bleeding after cardiac surgery has previously been reported,(1,2) but the role of inflammatory polymorphisms has not been determined. Objective: We tested the hypothesis that genetic polymorphisms of cytokines and cellular adhesion molecules are associated with bleeding and transfusion after cardiac surgery. Patients/Methods: 736 patients undergoing primary aortocoronary surgery with cardiopulmonary bypass were genotyped for 17 polymorphisms in 7 candidate genes : tumor necrosis factor, interleukins 1® and 6, interleukin 1 receptor antagonist, intercellular adhesion molecule -1 (ICAM-1), P-selectin and endothelial leucocyte adhesion molecule -1 (ELAM-1). Clinical co-variates previously associated with bleeding were recorded and DNA isolated from preoperative blood. Multivariable analyses were used to relate clinical and genetic factors to bleeding and transfusion.

Results: The 98G/T polymorphism of the ELAM-1 gene was independently associated with bleeding after cardiac surgery (p=0.002), after adjusting for significant clinical predictors (patient height and weight, and baseline hemoglobin concentration). There was a gene dose effect according to the number of minor alleles in the genotype; carriers of the minor allele bled 17% (GT) and 54% (TT) more than wild type (GG) genotypes, respectively (P=0.01). Carriers of the minor allele also had longer activated partial thromboplastin times (p=0.0023) and increased fresh frozen plasma transfusion (p=0.03) compared to wild type.

Conclusions: We found a strong dose-related association between the 98T ELAM-1 polymorphism and bleeding after cardiac surgery, independent of and additive to standard clinical risk factors. Testing for the 98T allele may be a useful preoperative stratification tool for risk of bleeding and transfusion.

Reference: 1)Welsby IJ et al. J Thromb Haemost 2005;3(6):1206-12. 2)Donahue BS et al. Circulation 2003;107(7):1003-8.



SCA40

MYOCARDIAL INJURY FOLLOWING CARDIOPULMONARY BYPASS WITH CARDIOPLEGIC ARREST IN THE RAT: DESCRIPTION OF A NOVEL MODEL

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Background: Cardioplegic arrest is an essential component of cardiac surgery but remains associated with a complex ischemia-reperfusion injury to the myocardium. Preclinical cardioprotection research has been limited so far to large animals or ex vivo heart preparations. The aim of this study was to investigate the degree of myocardial injury following cardiopulmonary bypass and cardioplegic arrest in a closed-chest rat model.

Methods: Male Sprague-Dawley rats (n=10 per group) were randomized to 3 groups: 1) cannulated without undergoing cardiopulmonary bypass (SHAM); 2) subjected to 75 min of non-pulsatile CPB as previously described (4)(CPB); 3) subjected to 75 min CPB including 30 min cardioplegic arrest (CA). In the CA group a 3.5 mm angioplasty balloon catheter was retrogradely inserted into the ascending aorta via the right common carotid artery and positioned under ultrasound guidance (SONOS 7500, Philips). After CPB onset the balloon was inflated to occlude the aortic lumen, 0.5 ml adult crystalloid cardioplegia solution was injected through the lumen, and cardiac arrest confirmed echocardiographically. Blood samples were taken at baseline (pre-CPB) and 1 hour post-CPB, and analyzed for cardiac fatty acid-binding protein (H-FABP) and troponin-I (TnI) using high-sensitivity ELISA. Serial echocardiographic examinations included two-dimensional and tissue Doppler imaging; measurements of systolic strain and strain rate were obtained in the anterior myocardium (Q-LAB software, Philips). Groups were compared using repeated measures ANOVA followed by Fishers exact test when appropriate, with $p < 0.05$ considered significant.

Results: Compared to SHAM and CPB groups, the CA group demonstrated significant increases in plasma biomarkers of myonecrosis (H-FABP and TnI) after CPB. Two-dimensional echocardiographic indices of global ventricular systolic function were not different between groups. However, significant reductions in peak systolic strain rate and ∑-ratio were observed in CA versus SHAM animals (Table 1).

Conclusion: We present the first comprehensive characterization of myocardial dysfunction and injury following cardiopulmonary bypass and cardioplegic arrest in the rat. This in vivo model is uniquely suited to further elucidate mechanisms of adverse myocardial outcomes following cardioplegic arrest and to evaluate novel cardioprotective strategies.

Table 1. Biochemical and echocardiographic indices of myocardial injury

	H-FABP (ng/ml)		Troponin I (ng/ml)		Peak systolic strain ratio		ε-ratio	
Group	Pre CPB	Post CPB	Pre CPB	Post CPB	Pre CPB	Post CPB	Pre CPB	Post CPB
CA	2.95(1.57)	13.86(8.84)*†	0.12(0.15)	9.54(4.77)*†	7.35(1.28)	4.85(1.18)*†	-	0.61(0.13)*
CPB	2.18(5.66)	5.66(3.46)†	0.14(0.19)	5.73(2.2)†	7.88(1.16)	6.43(0.88)	-	0.75(0.11)
SHAM	1.78(2.56)	2.56(2.30)	0.08(0.09)	3.46(2.29)†	7.43(1.61)	7.56(0.98)	-	0.95(0.05)

Data are expressed as mean(SD); H-FABP; Heart type fatty acid binding protein; ε - ratio; ratio of peak systolic strain post-CPB to pre-CPB; * $p<0.05$ versus SHAM, † $p<0.05$ versus pre-CPB

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SCA41

PROPOFOL REDUCES APOPTOSIS AND UP-REGULATES ENOS PROTEIN EXPRESSION IN HYDROGEN PEROXIDE STIMULATED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Propofol reduces apoptosis and up-regulates eNOS protein expression in hydrogen peroxide stimulated Human Umbilical Vein Endothelial Cells Authors: Baohua Wang PhD, Tao Luo MD, David Chen PhD, David M. Ansley MD Affiliation: University of British Columbia Dept of Anesthesiology, Pharmacology and Therapeutics, Dept of Chemistry, Vancouver, BC, CANADA

Introduction: Vascular endothelial cells play an important role in maintaining cardiovascular homeostasis. Oxidative stress is regarded as one of the critical pathogenic factors for endothelial cell damage and the development of cardiovascular diseases. The present study is designed to evaluate the protective effects of propofol on oxidative stress, induced endothelial cells insults, and their possible mechanisms.

Methods: Human umbilical vein endothelial cells (CRL1730) were used as the experimental model. Hydrogen peroxide (H2O2, 100?M) was used as the stimulus of oxidative stress. Study groups included 1) control cultured cells, 2) cultured cells incubated with only H2O2 3) cultured cells incubated with only propofol (50 ?M), or 4) cultured cells pre-treated with propofol 50 ?M for thirty minutes then co-incubated with H2O2. Cell viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the Trypan blue dye exclusion test. Cell apoptosis was evaluated by Hoechst 33258 staining. Activity of caspase-3 was determined by the colorimetric CaspACE Assay System (Promega, USA). Expressions of total Akt, phospho-Akt and endothelial nitric oxide synthase (eNOS) were detected by Western blotting.

Results: Exposure of endothelial cells to H2O2 decreased cell viability, induced cell apoptosis and increased caspase-3 activity. Propofol significantly protected cells from H2O2-induced cell damage and apoptosis. Propofol administration significantly decreased caspase-3 activity (119±17 % of control) as compared to H2O2 stimulated group (170±35 % of control). H2O2 did not significantly alter eNOS expression. Propofol treatment markedly increased eNOS expression as compared to control and H2O2 stimulated cells. However, there was no significant difference in phospho-Akt expression among the groups.

Conclusions: In the present study, we found that propofol protected endothelial cells from H2O2-induced damage and apoptosis, and induced eNOS up-regulation. We did not detect a difference in phospho-Akt expression between our groups. This contrasts with our recent findings that propofol increased phosphorylation of Akt in cultured myocytes (unpublished data). Endothelial nitric oxide (NO) produced by eNOS is capable of inhibiting apoptosis and is regarded as an endothelial cell survival factor 1. The phosphatidyl inositol 3-kinase (PI3K)/Akt pathway is able to regulate eNOS expression and activation 2. Our findings suggest that propofol may regulate eNOS expression in endothelial cells through an Akt-independent pathway.

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SCA42

GENE EXPRESSION SIGNATURES OF ATRIAL FIBRILLATION IN HUMAN LEFT ATRIAL MYOCARDIUM

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Introduction: Atrial fibrillation (AF) is a complex disease involving both genetic and environmental factors that result in deregulation of multiple mechanistic pathways (1). Most human molecular AF studies to date have used right atrial tissue; however, the mechanisms involved and their regulation are chamber-specific, with the left atrium playing a central role in the genesis of AF. The aim of this study was to analyze gene expression signatures in left atrial myocardium from patients in AF and normal sinus rhythm (NSR) and to develop signature-based predictions of rhythm state.

Methods: We performed microarray analysis on left atrial appendage tissue samples from 18 patients who underwent cardiothoracic surgery (12 with AF and 6 in NSR and no history of AF). Following standard RNA preparation and quality control, targets were hybridized onto Affymetrix HT-HGU133A microarrays with 22,216 probesets. Gene expression signatures that reflect the activity of deregulated myocardial pathways were identified using supervised classification methods of analysis previously described (2). The analysis selects a set of genes for which expression was most highly correlated with rhythm state (absolute value of Kendall's tau coefficient >0.6, $p<0.001$), and the dominant principal components from this set define the phenotype-related metagenes. The classification probability for each of the two possible rhythms was estimated by fitting a Bayesian binary regression model, and its predictive performance assessed by leave-one-out cross validation. Category overrepresentation analysis was used for specific gene ontology terms to identify biological pathways deregulated in AF (3).

Results: 125 probesets were selected as highly correlated with rhythm state in the training set. Probit binary regression using the first two metagenes yielded a model with a predictive accuracy of 94% as assessed by leave-one-out cross validation (Figure: lower left-AF, upper right-NSR patients). Among the genes providing predictive ability, apoptotic factors, adhesion molecules, complement activation, calcium regulation and ribosomal protein pathways were relatively overrepresented. Conclusion: We are reporting the application of gene expression signatures in human left atrial myocardium to identify pathway deregulation accurately predictive of rhythm states. Combined with ongoing similar efforts in patients with new onset postoperative AF, these findings provide insight into the mechanisms of initiation versus perpetuation of AF, help identify AF susceptibility genes, and may facilitate development of novel predictive and therapeutic strategies.

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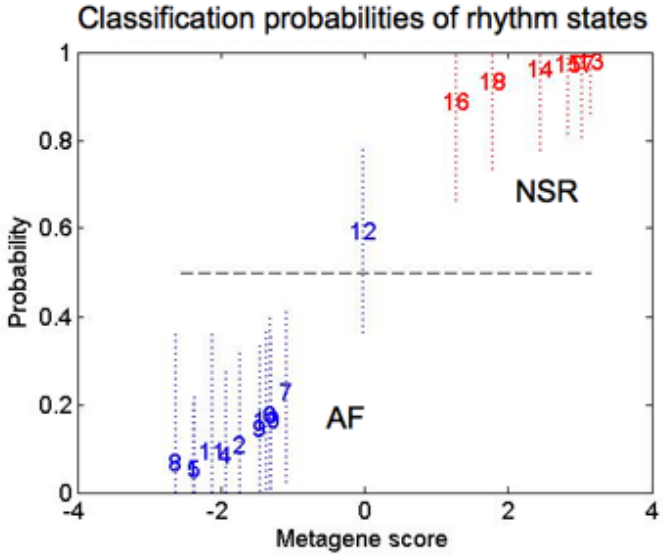

SCA43

MYOCARDIAL ENOS/INOS GENE AND PROTEIN EXPRESSION IN CABG PATIENTS RECEIVING LARGE DOSE PROPOFOL DURING ISCHEMIA AND EARLY REPERFUSION

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Introduction: Endothelial Nitric Oxide Synthase (eNOS) triggers enhanced nitric oxide production by inducible Nitric Oxide Synthase (iNOS) to confer cardioprotection (1,2). Propofol, in large dose, has been shown to attenuate biomarkers of oxidative stress and myocardial injury following ischemia-reperfusion. We tested the hypothesis that propofol modulates eNOS relative to iNOS in cardiac tissue as a possible mechanism to confer cardioprotection in patients undergoing coronary surgery with cardiopulmonary bypass.

Methods: Twenty-four patients scheduled for CABG surgery were randomized in a double blind study to receive isoflurane at 0.5 to 2% (n=12) or propofol at 1 mg/kg then 120 mcg/kg/min (n=12) for a treatment interval of 120 to 150 min (10 min prior to CPB until 15 mun after aortic declamping). Anesthetic and surgical management under normothermic conditions was standardized for both groups. Atrial tissue biopsies were obtained before the initiation of CPB and 15 minutes after reperfusion. We analyzed cardiac tissue for modulated expression of eNOS and iNOS. Gene expression changes were quantified by real-time RT-PCR while protein expression was assayed using Western blot anaysis.

Results: Patients were demographically similar in both groups. The pre- and post-bypass eNOS protein expression and mRNA levels in atrial tissue were not significantly different in patients treated with isoflurane ($P>0.05$). eNOS protein expression and mRNA levels did increase significantly from baseline in post-bypass atrial tissue from patients in the propofol treatment group ($P<0.05$). iNOS mRNA expression was consistently increased after bypass in atrial tissue from the propofol group. In contrast, iNOS gene expression was not appreciably different in atrial tissue derived from the isoflurane group. iNOS protein expression was undetectable in pre- and post-bypass atrial tissue biopsies regardless of anesthetic treatment.

Conclusion: Our data demonstrates that large dose propofol applied during cardiopulmonary bypass significantly upregulates myocardial eNOS protein and gene expression. We conclude that propofol increases myocardial nitric oxide bioavailability in CABG patients. The pattern of gene expression for eNOS relative to iNOS suggests that propofol may mediate delayed preconditioning of the myocardium (3). The mechanisms that yield this effect are currently unclear to us. Further investigation is required to determine whether these findings reflect the action of propofol alone, or a synergistic effect of propofol and isoflurane.

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SCA44

THE EFFECT OF APROTININ AND METHYLPREDNISOLONE ON NEUROLOGICAL OUTCOME AFTER DEEP HYPOTHERMIC CIRCULATORY ARREST IN THE RAT

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Introduction Although cardiac surgery with cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) has proven to be life-saving, prevention of its associated adverse sequelae remains a challenge [1]. Both, the cerebral ischemia-reperfusion injury associated with DHCA, along with the inflammatory reaction triggered by CPB appear to play an important role in the neurologic morbidity and related mortality. Aprotinin and steroids are both frequently used for their anti-inflammatory effects and are considered by some to have neuroprotective properties. The purpose of this investigation was to determine the effect of both these drugs on neurological outcome following experimental DHCA in the rat [2].

Methods After Duke Animal Care Committee approval, 30 male Sprague-Dawley rats (400-450g) were anesthetized and cannulated for CPB. The rats were randomly assigned to one of three groups (n=10 per group): 1) The Aprotinin group received 60,000 KIU/kg of aprotinin as a loading dose 15 minutes prior to CPB, followed by an infusion of 15,000 KIU/kg/h during CPB; 2) the Steroid group received 30 mg/kg of methylprednisolone 60 minutes prior to CPB; 3) the Sham-operated Controls received an equal amount of 0.9% NaCl. The rats were cooled to 18°C during CPB and when an isoelectric EEG was obtained, 60 minutes of DHCA was initiated. After rewarming to 35.5°C, the animals were separated from CPB and allowed to passively rewarm to 37°C. All rats were extubated 120 minutes after the end of CPB and recovered in an oxygen-enriched environment for 24 hours. At postoperative day 3 and 7, a blinded observer used a standardized neurological scoring system (0 indicates normal function; 48 is the maximum score) [3] to evaluate the rats. The neurological scores were compared with one-way analysis of variance with a $p< 0.05$ considered significant.

Results Compared to controls, neither aprotinin nor methylprednisolone had any significant neuroprotective benefit at day 3 ($p = 0.55$) or at day 7 ($p = 0.64$).

Conclusion Although 60 minutes of DHCA in the rat was associated with neurological dysfunction, neither aprotinin, nor steroids conferred any neuroprotection.

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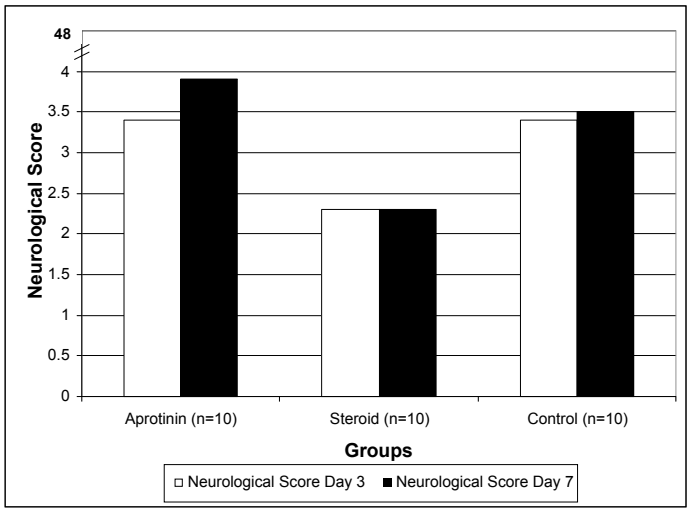

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SCA45

EFFECT OF CARDIOPULMONARY BYPASS ON EXPRESSION OF S100B AND RAGE IN THE RAT KIDNEY, HEART AND AORTA

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INTRODUCTION: Patients undergoing cardiac surgery with cardiopulmonary bypass are at high risk for the development of cardiac, neurological and renal complications. S100B is an intracellular regulatory protein which is released following cardiopulmonary bypass (CPB) and thought to be related to neurological injury; however there are other potential sources of this protein. S100B exerts its effects in part by binding to cell surface receptor for advanced glycation end-products (RAGE). Recently, S100B and RAGE have been shown as key modulators of cardiac injury. The purpose of this study was to determine the expression of S100B and RAGE in the tissues of rats undergoing CPB.

METHODS: With ACC approval, 12 Sprague-Dawley rats were anesthetized with ketamine, isoflurane, fentanyl, and propofol. Study rats underwent normothermic CPB for 1 hour with a neonatal membrane oxygenator. Sham animals were instrumented but did not undergo CPB. Animals were sacrificed at the end of CPB or at 6 hours after CPB and the following tissues were harvested: kidney (cortex and medulla), left ventricle (LV) and abdominal aorta. Quantitative real time RT-PCR and Western blotting was used to assess the expression of S100B and RAGE in each of the tissues.

RESULTS: There were no significant differences between groups in physiologic variables. S100B and RAGE were absent in the sham kidney and not induced following CPB. S100B was not detected in sham aorta and LV, but induced following CPB, up to 10 fold higher in LV tissue. RAGE showed a low level of basal expression in sham aorta and LV and following CPB, RAGE was similarly upregulated in both aorta and LV. There were no significant differences between 0 and 6 hours of CPB.

DISCUSSION: This study demonstrates that the heart and aorta are potential non-neurogenic sources of S100B and RAGE following CPB, and that these mechanisms may be involved in post-CPB cardiovascular dysfunction. The lack of expression of S100B and RAGE in the kidney suggests that they are unlikely to play an important role in the mechanisms of early renal dysfunction following CPB.

SCA46

APROTININ ANTAGONIZES TISSUE PLASMINOGEN ACTIVATOR-MEDIATED CELL TOXICITY ON CULTURED NEUROBLASTS

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Introduction: Serine proteases and their inhibitors play important roles in normal physiological homeostasis including neuronal activity, hemostasis, and wound healing.Tissue plasminogen activator (tPA) is involved in normal neuronal plasticity and memory formation but can also be neurotoxic [1].

Methods: Rat dopaminergic neuroblasts (1RB3AN27, or N27 cells) were used in all experiments.The cells were cultured in RPMI1640 (Sigma-Aldrich, MO) supplemented with 10 %(v/v) heat-inactivated fetal calf serum (Life Technologies, MD), 2mM glutamine, and 100 U/ml penicillin and 100 mg/ml streptomycin, 37°C in a humidified atmosphere of 95% air/5% CO2. Just before the commencement of experiments, the medium was changed to RPMI with 2% FCS. To fully eliminate possible effects of plasminogen-plasmin in serum, some experiments were repeated in serum free medium. Cells were exposed to tPA (10, 20 □g/ml) for 30,60, 120 min or 24 hrs with aprotinin (200KIU/ml) prior to, or after tPA exposure. The effects of the treatments were quantitated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, determining cell viability.

Results: We demonstrate that aprotinin, a serine protease inhibitor with antifibrinolytic and anti-inflammatory activities, blocked tPA-induced neurotoxicity. Treatment of cultured neuroblasts with tPA (10-20 □g/ml) caused a dose-dependent decrease of cell viability (Figure 1), which was accelerated in the absence of serum in the culture medium (data not shown). Aprotinin was effective in preventing cell death when administered 30 min prior to tPA exposure, but this protection was significantly less when aprotinin was administered after tPA and after long exposures to tPA (Figure 2).

Conclusion: The present data suggest that the reported neuroprotection [2] afforded by aprotinin may be due to inhibition of tPA-mediated neurotoxicity.

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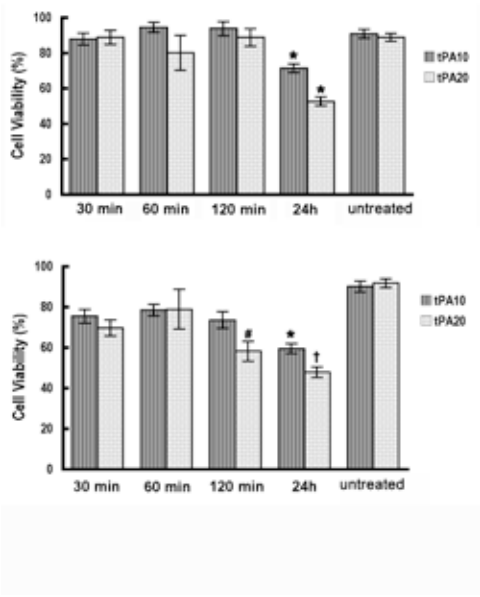
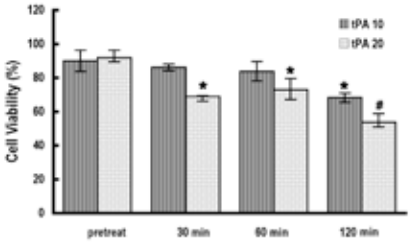
Figure 1.



N27 cell viability after tPA treatment (10 or 20 µg/ml) for various durations in 2 % serum (upper panel) and in serum-free media (lower panel). The percent (%) viability was calculated using MTT assay as a ratio of tPA-treated and untreated cells for each experiment. Data are shown as mean ± SE (n=8). *p < 0.05 versus untreated and tPA treatments (30, 60, 120 min) at respective tPA concentration, †p<0.05 versus untreated and tPA (20 µg/ml) treatments for 30 and 60 min, # p<0.05 versus untreated cells.

Figure 2.



Aprotinin reduces N27 cell death from tPA treatment in serum-free media. The cell culture was treated with aprotinin 30 minutes before (pretreat) or 30, 60, or 120 minutes after tPA exposure. The percent (%) viability was calculated using MTT assay as a ratio of tPA-treated and untreated cells for each experiment. Data are shown as mean ± SE (n=8). *p < 0.05 versus pre-treatment group at respective tPA concentration, #p< 0.05 versus other groups treated with tPA 20 µg/ml.

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SCA47

EFFECT OF CPB FLOW RATE ON CEREBRAL OUTCOME IN A RAT MODEL OF CARDIOPULMONARY BYPASS COMBINED WITH CEREBRAL AIR EMBOLI

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Introduction: Cerebral injury resulting in cognitive dysfunction, stroke, or death is an important complication after cardiac surgery with cardiopulmonary bypass (CPB)(1). Inadequate cerebral perfusion and cerebral embolization during CPB have been implicated as important factors in the etiology of this injury. However, the impact of CPB flow rate on cerebral outcome after CPB in the context of cerebral air emboli (CAE) has not been evaluated. Therefore, we hypothesized that alteration of CPB flow rate would affect functional and histologic outcome in a model of CPB and CAE(2).

Methods: With institutional animal care approval, male Sprague-Dawley rats were exposed to 90 min of normothermic CPB with 10 CAE (0.3 αl/bolus) injected repetitively via the right internal carotid artery. Rats were randomized to two groups (n=10 per group) that differed in regards to management of CPB flow rate during CPB: 1) CPB flow rate of 55-60 ml/min (high-flow), 2) CPB flow rate of 35-40 ml/min (low-flow). Mean arterial blood pressure (MAP) was kept constant throughout CPB at 50-60 mmHg. Cognitive function was evaluated using the Morris water maze test beginning on postoperative day (POD) 3 and continuing until POD7. Neurologic function (Neuroscore and Rotarod) was assessed on POD3 and 7 with cerebral infarct volumes determined after testing was completed.

Results: Rats managed with high CPB flow rates had lower (better) neurologic scores compared to those with low CPB flow rates on POD3 and 7 ($p<0.05$, figure 1). Moreover, rats managed with high CPB flow performed better in the Rotarod on POD3 but not on POD7 compared to those managed with low CPB flow ($p<0.05$). Cognitive function was not different between groups. Although subcortical and cortical infarct volumes were similar between groups, animals managed with high CPB flow rate had fewer infarcts than those with low CPB flow rate ($p<0.05$; figure 2). The number of infarcts correlated to neurologic scores on POD3 and 7 ($p<0.02$).

Conclusion: Utilizing a rat model of CPB, this study demonstrates the beneficial effects of maintaining higher CPB flow rates on postoperative neurologic function when a fixed gaseous embolic load enters the cerebral circulation. This functional benefit was associated with lower numbers of cerebral infarcts.

References: (1). Roach GW, et al. N Engl J Med 1996;335:1857-1863. (2). Jungwirth B, et al. Anesthesiology 2006;104:770-776.

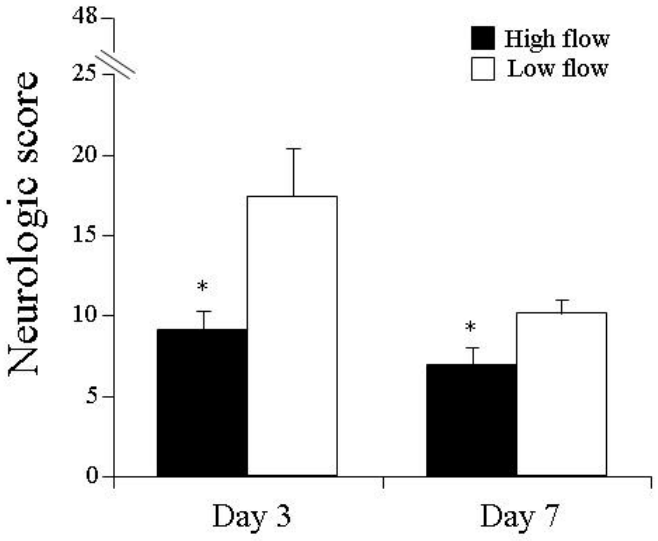

Figure 1. Results are shown as Mean ± SEM
* $p< 0.05$ between both groups.

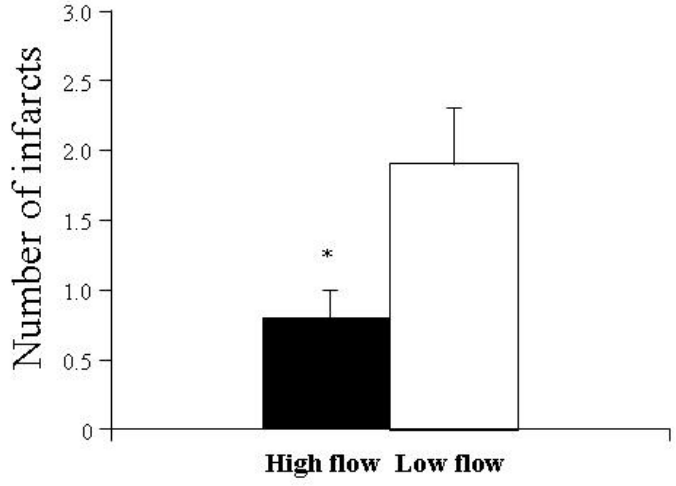

Figure 2. Results are shown as Mean ± SEM
* $P < 0.05$ between both groups.

SCA48

APROTININ RESULTS IN ACUTE RENAL INJURY AFTER DEEP HYPOTHERMIC CIRCULATORY ARREST IN THE RAT.

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Objective: Deep hypothermic circulatory arrest (DHCA) is associated with an increased risk of perioperative renal dysfunction leading to considerable morbidity and mortality. The ischemia-reperfusion injury related to DHCA along with the inflammatory reaction associated with cardiopulmonary bypass (CPB) may play an important role in the etiology of renal dysfunction. As both aprotinin and methylprednisolone are frequently used for their anti-inflammatory properties in this setting, we sought to determine the effects of these drugs on renal injury after experimental DHCA.(1)

Methods: After induction of anesthesia, male Sprague-Dawley rats were cannulated for CPB and DHCA. Rats were randomly assigned to one of three groups (n=10 per group): 1) rats in the aprotinin group received 60,000 KIU/kg of aprotinin as a loading dose 10 min prior to CPB, followed by an infusion of 15,000 KIU/kg/h during CPB; 2) animals in the steroid group received 30 mg/kg of methylprednisolone 10 min prior to CPB; and 3) sham operated controls received an equal volume of 0.9% NaCl. During CPB, animals were cooled to 18°C and exposed to 60 min of DHCA. Following rewarming, the rats were extubated 120 min after bypass and recovered in an oxygen enriched environment for 24 hrs. At day 7, rats were sacrificed and both kidneys were removed, fixed in formalin, following which they were sectioned and stained with hematoxylin and eosin allowing for histological assessment. A blinded observer assessed the renal injury in 50 proximal renal tubules from each kidney using a previously described tubule histopathology score (accordingly: 0 - no ischemic damage; 1 - mild ischemic damage; 2moderate ischemic damage, 3 - severe ischemic damage).(2) Mean tubule histopathology scores were compared among the three groups using the Kruskal Wallis test, which when significant, was followed by post hoc testing using the Mann Whitney U statistic.

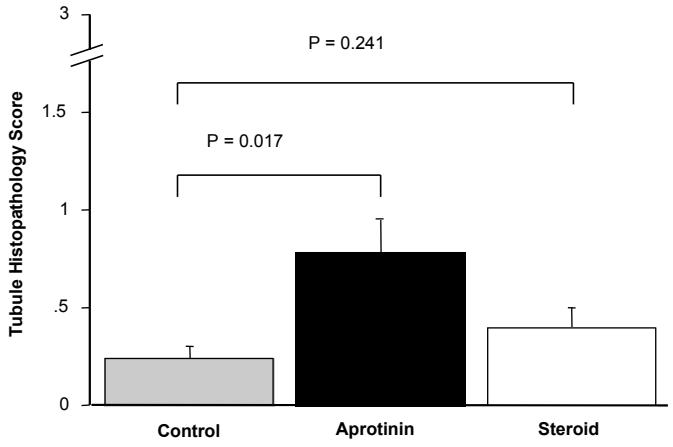
Results: 30 rats (10 in each group) completed the study and their kidneys were evaluated for ischemic injury. Compared to the control group, the renal tubules in the aprotinin group showed significantly more ischemic injury (p = 0.017; see Figure). There was no difference between the control and steroid groups.

Conclusions: The use of aprotinin during DHCA was associated with an increase in ischemic injury to the renal proximal tubules. Methylprednisolone had no effect on renal tubular injury.

References:

1 Jungwirth B, Mackensen GB, Blobner M et al. Neurologic outcome after cardiopulmonary bypass with deep hypothermic circulatory arrest in rats: description of a new model. J Thorac Cardiovasc Surg 2006;131:805-12.

2 Hamar P, Song E, Kokeny G et al. Small interfering RNA targeting Fas protects mice against renal ischemia-reperfusion injury. Proc Natl Acad Sci USA 2004;101:14883-8.



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SCA49

INJURY-SPECIFIC MYOCARDIAL GENE EXPRESSION SIGNATURES FOLLOWING CARDIOPULMONARY BYPASS AND CARDIOPLEGIC ARREST IN THE RAT

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Introduction: Ischemia-reperfusion and inflammation modulate severity of myocardial injury associated with cardiac surgery. We have previously reported myocardial activation of ischemia-independent proinflammatory gene cascades in response to CPB-specific injury in a closedchest model of rat CPB. The aim of this study was to investigate patterns of deregulated myocardial gene expression in a novel model of rat CPB and cardioplegic arrest that mimics the full spectrum of cardiac surgery-related injuries, and to evaluate the contribution of each mechanism.

Methods: Male Sprague-Dawley rats were randomized to 3 groups (n=5 each): cannulated without undergoing CPB (Sham); 75 min of mild hypothermic (33C) CPB (CPB); 75 min of mild hypothermic CPB with 30 min cardiac arrest using crystalloid cardioplegia (CA). Total RNA from left ventricular myocardial tissue harvested 1h after CPB was hybridized to Rat Operon3.0 microarrays, containing 27,648 features. Gene expression signatures reflecting responses to each category of environmental perturbation were identified using supervised classification methods of analysis as described (1). First, a set of genes was selected for which expression levels were most highly correlated with the experimental groups (Kendalls tau $p<0.05$). The dominant principal components from this set of genes define the phenotype-related metagenes. The classification probability for each of experimental group was estimated by fitting Bayesian binary regression models, and their predictive performance assessed by leave-one-out cross validation. Category overrepresentation analysis was used for specific gene ontology terms to identify biological pathways deregulated in response to CA and CPB.

Results: As expected, both CPB and CA resulted in robust changes in myocardial transcriptome (Fig 1). Probit binary regression using the first two metagenes yielded models with predictive accuracies of 90% as assessed by leave-one-out cross validation (Fig 2). Icosanoid metabolism, mitochondrial membrane biogenesis, regulation of fibroblast proliferation, alkene metabolism, Hedgehog signaling, proteolysis and B-cell receptor signaling were pathways overrepresented in the CA vs CPB groups. On the other hand, regulation of apoptosis (both pro and anti) and immune response pathways were activated in common by CPB and CA.

Conclusions: The successive interventions associated with clinical cardiac surgery result in specific pathway deregulation that can be identified using myocardial gene expression profiling. Such myocardial injury signatures may be used to guide targeted cardioprotective therapeutics.

1. West M, et al. Proc Natl Acad Sci 2001

Fig.1 Venn diagram of differentially expressed myocardial genes by experimental group

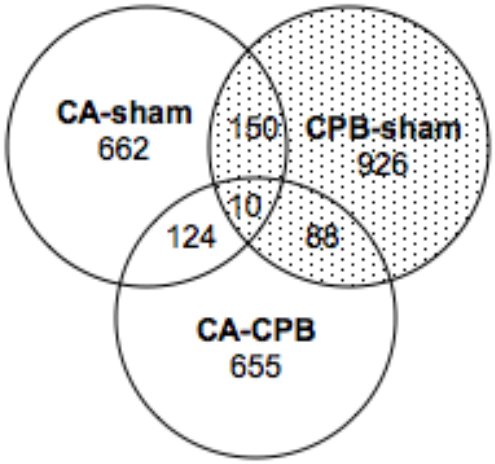

Figure 2