

SCA37

NORMOTHERMIC CARDIOPULMONARY BYPASS REDUCES DIAPHRAGM CONTRACTILE FORCES IN RATS

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Objective: Diaphragm dysfunction has been described as a potential complication of cardiac surgery requiring cardiopulmonary bypass (CPB). This is often attributed to hypothermia and topical cooling (1, 2). Here, we assessed the effect of cardiopulmonary bypass on diaphragm muscle contractile forces under normothermic conditions in rats.

Methods: Ten male Sprague Dawley rats (body weight: ~ 450 g) were randomly assigned in two groups. The sham control group (Sham) underwent mechanical ventilation and cannulation without CPB. The experimental group (CPB) underwent cardiopulmonary bypass for one hour followed by a 90 minute recovery period. CPB was performed using a modified technique based on a previously reported model (3). The right internal jugular vein and left carotid artery were used as the venous and arterial cannulation sites. CPB was applied using a miniature extracorporeal circuit composed of non-blood prime (14 ml) and conducted at 37°C using a heat exchanger integrated with the venous reservoir. A Plexiglas encased membrane oxygenator was employed, and a mini-ECMO pump was used at flow rates of approximately 30 ml/min, maintaining mean arterial blood pressure at 60 mmHg. Diaphragm contractile force measurements were performed using established protocols (4). Briefly, midcostal hemidiaphragm muscles with phrenic nerves were excised. A muscle segment (810 mm wide) was used for isometric twitch force (Pt) and maximum tetanic force (Po) measurements. Muscle fatigue resistance was assessed using repetitive stimulation at 40 Hz in trains of 330 ms duration repeated once each second. Fatigue index was calculated as the ratio of force generated after 2 minutes of stimulation to the initial force.

Results: Baseline characteristics and hemodynamics were similar between the two groups. All rats in the CPB group survived and tolerated the CPB and recovery period. The twitch force and maximal tetanic force were significantly reduced in the CPB group (Pt: 8.4 vs. 5.9 N/cm², p = 0.0002; Po 16.9 vs. 11.5 N/cm², p = 0.0001, in Sham vs. CPB, respectively). There was no difference in the muscle fatigue index between the two groups.

Conclusion: These data demonstrate that cardiopulmonary bypass, without hypothermia or topical cooling, has deleterious effects on diaphragm muscle function. Diaphragm skeletal muscle exhibits worsened

contractile properties after CPB in the rat. These findings may contribute to the physiologic mechanisms underlying diaphragm dysfunction in cardiac surgical patients.

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SCA38

INHIBITION OF APOPTOTIC PROTEIN P53 LOWERS THE THRESHOLD FOR HELIUM-INDUCED CARDIOPROTECTION IN RABBITS

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Introduction: Brief, repetitive administration of helium (He), a noble gas without anesthetic properties, before prolonged coronary artery occlusion and reperfusion protects myocardium against infarction by activating phosphatidylinositol-3-kinase (PI3K) signaling and inhibiting the mitochondrial permeability transition pore (mPTP) (1). PI3K stimulates degradation of p53, a protein that produces apoptosis by facilitating mPTP opening (2). Whether selective inhibition of p53 enhances the protective effects of He is unknown. We tested the hypothesis that inhibition of p53 lowers the threshold of He cardioprotection via a mPTP-dependent mechanism.

Methods: Rabbits were anesthetized with sodium pentobarbital (30 mg/kg), acutely instrumented for the measurement of systemic hemodynamics, and ventilated using positive pressure with an air-oxygen mixture ($FiO_2 = 0.30$). All rabbits were subjected to a 30 min left anterior descending coronary artery (LAD) occlusion followed by 3 h reperfusion. Rabbits ($n=7$ to 8 per group) were randomly assigned to receive 0.9% saline (control), or one, three, or five cycles of 70% He-30% oxygen administered for 5 min interspersed with 5 min of the air-oxygen mixture before coronary artery occlusion. Additional rabbits received the selective p53 inhibitor pifithrin- α (PIF; 1.5 or 3.0 mg/kg) or the combination of PIF (1.5 mg/kg) plus He (one cycle) before LAD occlusion in the presence or absence of the mPTP opener atractyloside (5 mg/kg). Myocardial infarct size was determined using triphenyltetrazolium chloride staining. Statistical analysis was performed with analysis of variance followed by Bonferroni's modification of Student's t-test.

Results: Systemic hemodynamics, arterial blood gas tensions and acid-base status, and arterial oxygen saturation (pulse oximetry) were unchanged during administration of He with or without PIF. Body weight, LV mass, area at risk weight, and the ratio of area at risk to LV mass were similar between groups. One, three, and five cycles of helium significantly ($P<0.05$) reduced myocardial infarct size [35 ± 6 , 25 ± 4 , and 20 ± 3 , respectively, of left ventricular area at risk (mean \pm SD)] compared with control ($44\pm 6\%$). PIF alone (3.0 but not 1.5 mg/kg) also reduced myocardial necrosis (24 ± 1 , and $46\pm 2\%$, respectively). Pretreatment with the combination of 1.5 mg/kg PIF and one cycle of He decreased infarct size ($22\pm 2\%$) to an equivalent degree as three cycles of He pretreatment alone. The cardioprotective effects of combined administration of PIF and He were abolished by atractyloside pretreatment ($47\pm 2\%$).

Conclusions: The results demonstrate that He-induced protection against myocardial infarction is dose-dependent and further indicate that inhibition of apoptotic protein p53 lowers the threshold of He-induced preconditioning in vivo.

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SCA39

THE EFFECT OF TEMPERATURE AND MINOCYCLINE ON OUTCOME AFTER PROLONGED HYPOTHERMIC CIRCULATORY ARREST IN RATS

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Introduction: Conventional resuscitation of exsanguination cardiac arrest (CA) victims is generally unsuccessful. Over 50% of deaths due to trauma occur at the scene, where medical care is limited. However, in an appropriate setting, some of those traumatic injuries could be surgically repairable. Emergency preservation and resuscitation (EPR) is a novel approach that uses an aortic flush to induce deep hypothermia during CA, followed by delayed resuscitation with cardiopulmonary bypass (CPB). Since deep hypothermia takes time to induce, agents that augment its benefit are needed. Minocycline is neuroprotective in a number of brain injury models via attenuating microglial activation. Hypothesis:

Hypothermia and minocycline would improve outcome after exsanguination CA in rats. **Methods:** Isoflurane anesthetized rats were subjected to a lethal hemorrhage (12.5 ml over 5 min). After 5 min of no-flow, hypothermia was induced via aortic flush. Three groups were studied: room-temperature (RT) saline flush, ice-cold (IC) flush and RT flush followed by minocycline (M, 20 mg/kg iv at 1 h and 90 mg/kg ip at 24 and 48 h). After 20 min of CA, resuscitation was achieved via CPB. Survival, Overall Performance Category (OPC, 1=normal, 5=death), Neurologic Deficit Score (NDS, 0-10%=normal, 100%=max deficit), neuronal death and microglial activation in hippocampus were assessed at 72 h using Fluoro-Jade B (FJB) and Iba-1 staining, respectively.

Results: Rats in the IC group had lower tympanic temperature during CA vs other groups (RT 28.4±0.6 °C; IC, 20.9±1.3 °C; M, 28.3±0.7 °C; p<0.001). While survival was similar in all groups (RT, 6/9; IC, 6/7; M, 6/11), neurological outcome was better in the IC group vs other groups (OPC: RT, 3±1; IC, 1±1; M, 2±1; p<0.05; NDS: RT, 55±19%; IC, 8±9%; M, 27±16%; p<0.05). Neuronal death in CA1 and dentate gyrus was similar in all groups (p=0.15), but microglial activation was attenuated in the IC group vs all other groups (p<0.01) (Table).

Conclusions : Deeper levels of hypothermia induced by the IC vs RT flush resulted in better neurological outcome in survivors. Surprisingly, hypothermia attenuated microglial activation but not hippocampal neuronal death. Minocycline had modest benefit on neurologic outcome in survivors, but did not attenuate microglial activation in brain. Our findings suggest a novel effect of hypothermia on microglial activation during prolonged circulatory arrest.

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Table. Functional outcome, neuronal death and microglial activation after 20 min CA. OPC 1=normal, OPC 2=mild disability, OPC 3=moderate disability, OPC 4=severe disability, OPC 5=death. Each dot represents one rat. Numbers represent mean±SD. *p<0.01 vs. other groups.

Group	RT	IC	M
Flush	room-temp	ice-cold	room-temp
Minocycline	no	no	yes
Neuro outcome			
OPC 5	•••	•	•••••
OPC 4			
OPC 3	••••		••
OPC 2	••	••	•••
OPC 1		••••	•
Histo outcome			
FJB+ neurons	27±15	15±8	26±13
Iba-1+ microglia	78±38	31±14*	103±36

SCA40

PITX2 REGIONAL GENE VARIANTS ARE ASSOCIATED WITH NEW-ONSET ATRIAL FIBRILLATION AFTER CARDIAC SURGERY

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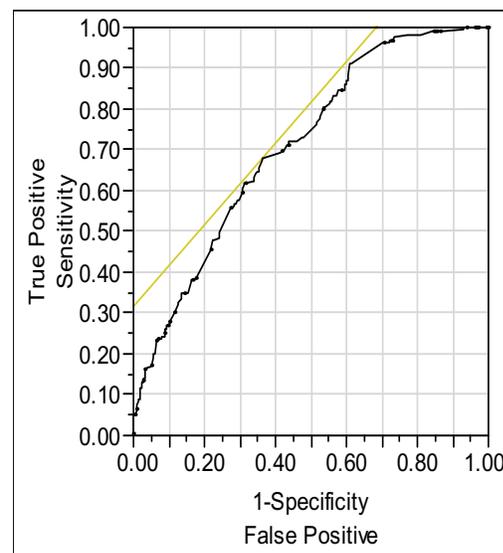
Introduction: Atrial fibrillation (AF) is the most-common adverse event after cardiac surgery. AF is associated with longer hospital stays, increased cost, and long-term mortality. Variants of chromosomal region 4q25, in proximity to the paired-like homeodomain transcription factor 2 (PITX2) gene, have been associated with development of AF in ambulatory populations. PITX2 is a transcription factor that regulates procollagen lysyl hydroxylase gene expression. This protein is thought to play a role in left-right determination and development of the embryonic atria. We investigated the role of 4q25 variants in development of new-onset AF after cardiac surgery.

Methods: 34 haplotype-tagging single nucleotide polymorphisms (SNPs), encompassing the 4q25 region previously associated with AF, were genotyped in 646 Caucasians undergoing cardiac surgery at two institutions. After applying genotyping quality control filters, 32 SNPs were examined by multivariate logistic regression for association with postoperative new-onset AF, adjusting for patient demographics, perioperative risk factors, and medications. Identified associations were also adjusted for multiple comparisons by permutation.

Results: 27.9% of patients experienced postoperative AF. Inheritance of one or more minor alleles (A) of dbSNP rs2200733 was associated with postoperative AF (risk ratio = 1.47; 95% confidence interval = 1.12 1.92; P = 0.008). After adjustment for patient age, gender, race, institution, postoperative use of b-blockers, statins and other previously identified predictors of AF, the A allele continued to be associated with postoperative AF (risk ratio = 1.42; 95% CI = 1.14 1.77; P = 0.002). Other SNPs in strong linkage disequilibrium with rs2200733 showed similar significant association with postoperative AF.

Conclusion: Expression of the minor allele of SNP rs2200733, lying approximately 170 kb base pairs from PITX2, is an independent predictor of new onset postoperative AF after cardiac surgery. This same SNP has been previously identified as a predictor of AF in ambulatory populations, and has the strongest correlation with postoperative AF amongst SNPs in the 4q25 region in this cohort.

Figure 1: Receiver operating characteristic for rs2200733 predicting new-onset postoperative AF for the clinical and genetic model. Area under curve = 0.710



SCA41

DOES THE PERIOPERATIVE APPLICATION OF MOXIFLOXACIN INFLUENCE LONG TERM NEUROLOGIC AND HISTOLOGIC OUTCOME FOLLOWING DEEP HYPOTHERMIC CIRCULATORY ARREST IN RATS?

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Introduction: Neurologic deficits following cardiac surgery with cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) continue to affect both quality of life as well as overall health-care costs. Inflammatory reaction is discussed as one potential contributor to these adverse outcomes and consequently as potential approach for neuroprotective drugs. In this context the antibiotic moxifloxacin (MXF) has been shown to reduce infarct volume after focal ischemia in mice (1) and to improve cerebral histologic outcome one day after DHCA in rats (2). The current study was designed to investigate the influence of MXF on long term neurologic and histologic outcome after DHCA in rats.

Methods: With IRB approval, 50 rats were randomly assigned to one of three groups: rats of the DHCA group were connected

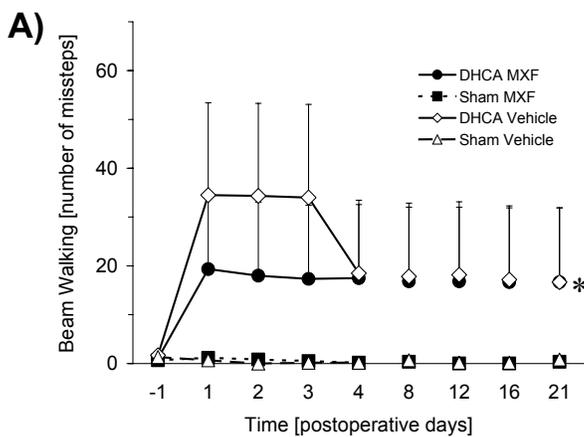


Figure A) shows neurologic function assessed as time balanced on a beam preoperatively and 21 days following CPB with DHCA. * = $p < 0.05$ vs. Sham

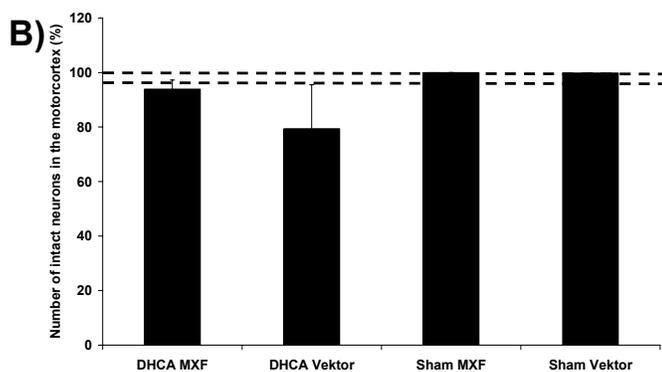


Figure B) depicts the number of intact neurons (%) in the motorcortex 21 days following CPB with 45min of DHCA (means \pm standard error). Depicted between dashed lines is the 95% confidence interval of untreated control animals.

to CPB, cooled to a rectal temperature of 15-18°C (30 min), exposed to 45 min of DHCA, followed by rewarming over 40 min to 35.5°C. Sham animals were anesthetized and surgically cannulated accordingly but not connected to CPB. Untreated animals served as control. Groups were further subdivided into a MXF and a Vehicle group receiving either MXF (6x100 mg/kg i.p.) or NaCl every 2 hours with the onset of anesthesia. Neurologic outcome was assessed pre- and 21 days postoperatively as time balanced on a beam. The percentage of undamaged neurons was assessed in the hippocampus CA1-region and in the motorcortex using HE staining. Data were analyzed using ANOVAs with post hoc Bonferroni ($p < 0.05$).

Results: Neurologic function was not impaired in the sham-operated rats but in both DHCA groups until postoperative day 21 (fig. A). Compared with untreated controls, both DHCA groups showed reduced numbers of intact neurons in the motorcortex but not compared to the Sham groups (fig. B). The number of intact neurons in the hippocampus CA1-region, was reduced in the DHCA Vehicle group, while the remaining three experimental groups demonstrated normal hippocampal histology (fig. C).

Conclusions: The perioperative application of MXF leads not only to a short-term, but also to a long-term improvement of histologic outcome in the hippocampus after DHCA in rats. However, MXF does not improve gross neurologic function or histologic outcome in the motorcortex. Consequently, the investigation of neurocognitive performance in this context would be of particular interest, as this function substantially depends on the integrity of the hippocampus.

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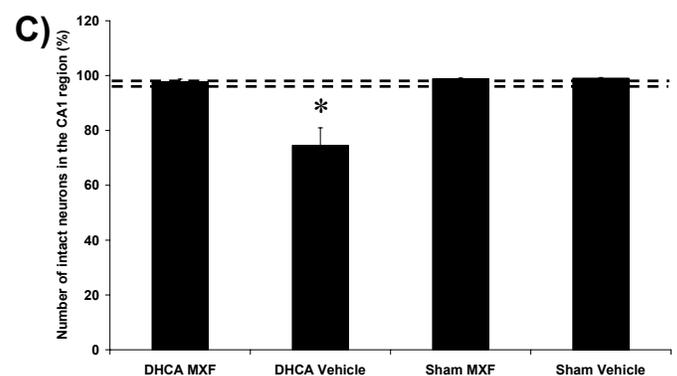


Figure C) shows the number of intact neurons (%) in the hippocampus' CA1 region 21 days following CPB with 45min of DHCA (means \pm standard error). Depicted between dashed lines is the 95% confidence interval of untreated control animals. * = $p < 0.05$

SCA42

GENE EXPRESSION SIGNATURES OF CEREBRAL INJURY FOLLOWING DEEP HYPOTHERMIC CIRCULATORY ARREST IN NEONATAL PIGS

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Objective: Despite improvements in overall mortality and morbidity of children undergoing surgical repair of congenital heart disease with deep hypothermic circulatory arrest (DHCA), incidence of neurological and neurodevelopmental dysfunction has changed little over the last decade and mechanisms of DHCA-associated injury in the newborn brain remain poorly understood. We aimed to investigate patterns of deregulated cortical gene expression associated with cerebral injury in a neonatal model of cardiopulmonary bypass (CPB) and DHCA.

Methods: Newborn piglets were assigned to sham, hypothermic CPB, or DHCA (n=3 each). DHCA and CPB animals were placed on full bypass, cooled to 18°C, underwent 60 min of DHCA or hypothermic CPB respectively, rewarmed to normothermia, weaned from CPB, and recovered for 30 minutes. Total RNA extracted from homogenized cerebral neocortex was hybridized to porcine microarrays. Differential gene expression for each pair of experimental conditions was evaluated based on $p < 0.01$ by linear models. The dominant principal components from these sets of genes define the phenotype-related metagenes used to estimate classification probabilities for each experimental group by fitting Bayesian binary regression models, and their predictive performance assessed by leave-one-out cross-validation. (1) Category overrepresentation analysis used for specific gene ontology terms identified biological pathways deregulated in response to DHCA and CPB (Ingenuity Pathway Analysis).

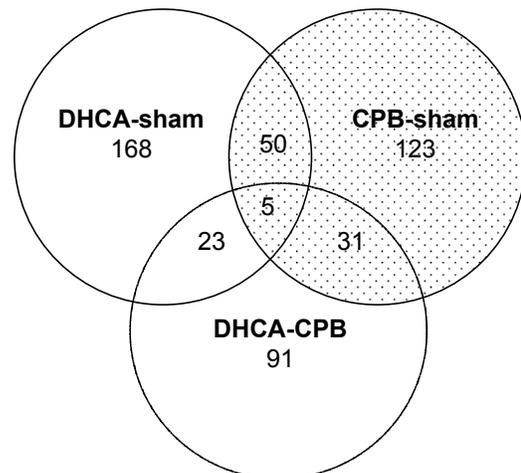
Results: CPB-DHCA resulted in extensive histological and ultrastructural cerebral injury, as well as robust changes in cortical gene expression (Figure). Probit binary regression using the first two metagenes yielded models with classification accuracies $>90\%$. Top canonical pathways significantly deregulated in response to hypothermic CPB included PI3K/AKT, glucocorticoid receptor, urea cycle, and dopamine receptor signaling. In contrast, DHCA resulted in further deregulation of glucocorticoid receptor, PPAR, NRF2-mediated oxidative stress response, neurotrophin/TRK, p53, protein ubiquitination, arginine-proline metabolism, and proapoptotic signaling pathways. Real-time polymerase chain reaction validated microarray results for the top upregulated and downregulated genes (*Pomc* and *Apoa1*, respectively).

Conclusions: Using whole-genome expression analysis, we characterized acute pathway deregulation associated with global cerebral ischemia-reperfusion in a neonatal model of CPB-DHCA. Such tissue-specific molecular signatures may be used to identify biomarkers of cerebral injury and guide development of targeted perioperative neuroprotectants. One example is apolipoprotein A1, a negative acute phase reactant, for which the observed transcriptional changes in response to DHCA corroborate with similar changes at the protein level in both brain and plasma. (2)

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Fig. Venn diagram of differentially expressed cerebral genes by experimental group



Recent data indicate that pulmonary resection during single lung ventilation (SLV) elicits a change in myocardial calcium cycling due, at least in part, to inhibitory nitrosative modification of the sarcoplasmic endoreticular calcium ATPase subtype 2a (SERCA2a) (1). Aging has been associated with similar modification of SERCA2a and, in some studies, decreased SERCA2a expression, resulting in impaired myocyte calcium cycling (2,3). The present study was designed to test the hypothesis that advanced age augments the decline in SERCA2a activity induced by pulmonary resection during SLV.

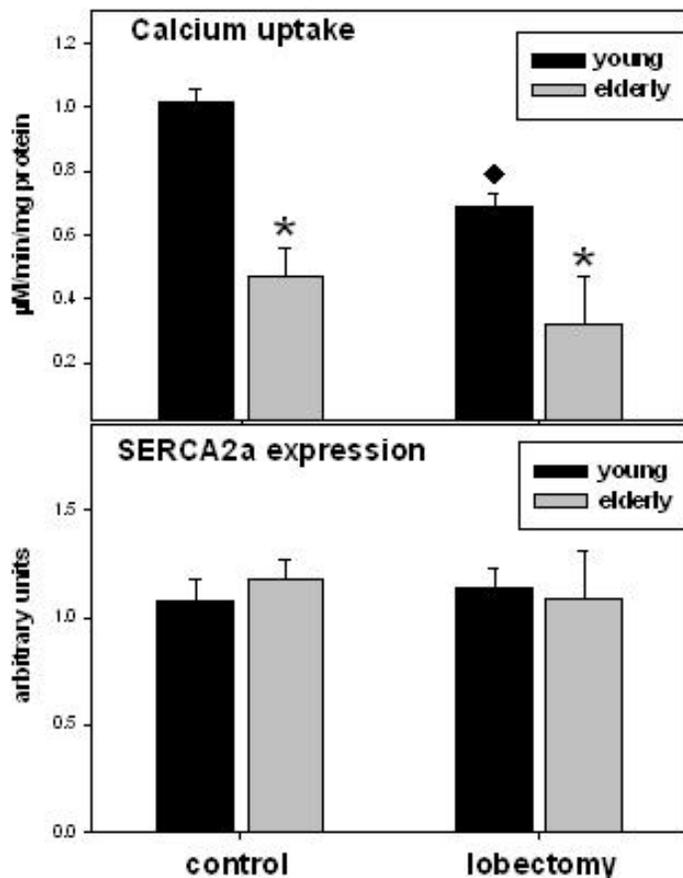
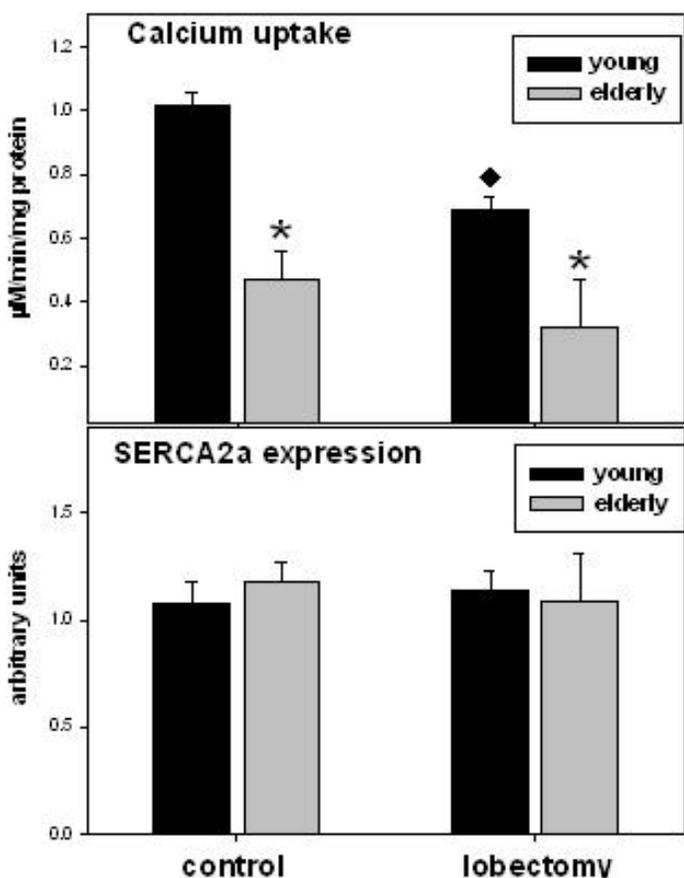
Methods: Myocardium harvested from 20 Sinclair swine were used for the study. Ten animals were young adults (~1 year of age) and 10 elderly animals (>10 years of age with an expected life span of 12 years). Within each age group, animals had either undergone left upper lobectomy during SLV (5 young, 5 elderly with tissue obtained on the third postoperative day) or were non-operated controls (5 young, 5 elderly). Sarcoplasmic reticular vesicles (SR) were prepared from each sample and analyzed for SERCA2a-mediated calcium uptake (Indo-1) and SERCA2a protein expression by Western blotting. Data within and between groups were compared by ANOVA with p<0.05 considered significant.

Results: As shown in figure panel A (data are mean +/- SE), lobectomy in young animals was associated with a nearly 40% decline in SR calcium uptake. Relative to young control swine, SR calcium uptake in elderly animals was reduced by 60%. However, in contrast to young animals, lobectomy was not associated with a lower SR calcium uptake relative to control in elderly swine. As shown in figure panel B, neither age nor lobectomy had any influence on SERCA2a expression.

Conclusions: The data indicate that in Sinclair swine, advanced age is associated with a marked decline in SERCA2a activity that cannot be attributed to a change in protein expression. However, the data do not support the study hypothesis in that the relative difference in SERCA2a activity between control and lobectomy animals was much greater for young than elderly swine. Overall, these findings support the probability that following lobectomy SERCA2a activity is altered by modification of the protein, and suggest that pre-existing age-related changes in SERCA2a structure prevent further perioperative modification.

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SCA44

A COMPARISON OF GENE EXPRESSION CHANGES IN PERIPHERAL CIRCULATING LEUKOCYTES AND CEREBRAL TISSUE IN RESPONSE TO CPB/DHCA IN RATS

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Background: Perioperative cerebral injury (PCI) complicating major cardiovascular surgery remains poorly predicted by clinical and procedural risk factors. In an established rodent model of cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) we compared genomic signatures in hippocampal tissue and peripheral blood leukocytes (PBL) in response to CPB and DHCA.

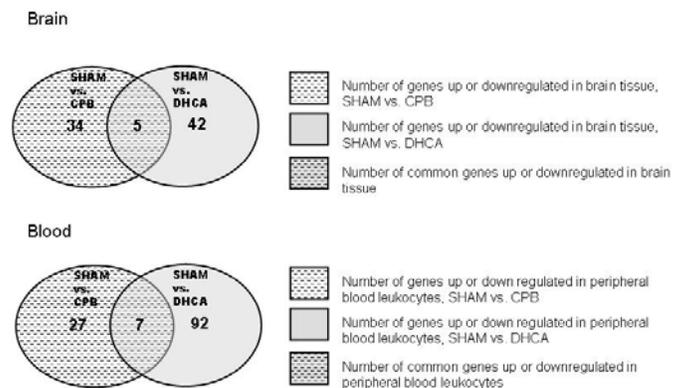
Methods: Male Sprague-Dawley rats subjected to CPB only (120 min), CPB (60 min) with DHCA (60 min), or sham surgery (n=3 each) were recovered for 24 hrs. Microarray profiling (Rat Operon 3.0; 27,648 sequences) was conducted using total RNA from hippocampus and PBL. Hippocampal and PBL injury genomic classification models were constructed individually using shotgun stochastic search approach and Bayesian model averaging, and their predictive accuracies tested by five-fold cross validation.

Results: Robust deregulation of gene expression in a stimulus and tissue-specific manner was observed and is displayed in the Venn diagram (Figure 1). Analysis was based on 12,033 gene sequenc-

es following removal of sequences with missing data. Among the 27 hippocampal genes deregulated in response to DHCA versus Sham, 16 genes were upregulated and 11 downregulated. Respectively, 37 genes were upregulated and 55 downregulated in response to DHCA versus Sham in PBL. Classification accuracies of blood-based expression signatures were comparable to those derived from the hippocampus. However there was no overlap between deregulated genes in the PBL and the brain. Top differentially expressed pathways included GM-CSF (upregulated) and B-cell receptor signaling (upregulated) in the brain and protein ubiquitination (down-regulated) and peroxisome proliferator-activated receptor (PPAR) pathway in PBL.

Conclusions: Perfusion techniques associated with major cardiovascular surgery such as CPB and DHCA induce changes in leukocyte gene expression that can predict the spectrum of experimental conditions, with potential applications in perioperative risk stratification and selection of targeted neuroprotective strategies.

Figure 1



SCA45

L-ARGININE COMPROMISES PROPOFOL PROTECTION OF THE ENDOTHELIUM AGAINST TNF-ALPHA INDUCED APOPTOTIC CELL DEATH

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Background: Nitrate tolerance (NT), a phenomenon where the clinical or hemodynamic response to organic nitrates is attenuated or abolished after prolonged, continuous or high dose nitrate treatment, has been shown to aggravate postischemic myocardial apoptosis and impair myocardial functional recovery. The mechanism governing NT aggravation of post-ischemic myocardial injury is not very clear. Vascular endothelial cell apoptosis has been shown to precede cardiomyocyte apoptosis during myocardial ischemia-reperfusion, a situation that is associated with increased tumor necrosis factor- α (TNF) production. We thus postulated that nitrate treatment may exacerbate TNF induced endothelial cell apoptosis, and compromise the protective effects of propofol, an intravenous anesthetic with antioxidant property that has been shown to attenuate TNF induced endothelial cell apoptosis.

Methods: L-arginine, a nitric oxide precursor, was used as the nitrate supplier. Cultured human umbilical vein endothelial cells (HUVECs) (cell line ECV304) were either not treated (control), or treated with TNF (40 ng/mL) (T) alone or TNF in the presence of 50 μ M propofol (T+P), 100 μ M of L-arginine (T+LA), 100 μ M of the non-selective nitric oxide synthase inhibitor N (ω -nitro-L-arginine (L-N) (T+LN), 250 μ M of an inducible nitric oxide synthase (iNOS) inhibitor aminoguanidine (T+A), propofol plus L-arginine (T+P+LA) or propofol plus L-NNA (T+P+LN), respectively, for 24 hr. Cell apoptosis was determined using flow cytometry. The generation of superoxide and hydrogen peroxide was assessed by dihydroethidium and dichlorofluorescein fluorescence staining, respectively.

Results: The apoptotic index (100% of apoptotic cells) was 1.5 \pm 0.6% (control), 36.9 \pm 2.4% (T, P<0.01 vs. Control), 27.8 \pm 1.2% (T+P, P<0.01 vs. T), 44.9 \pm 1.4% (T+LA, P<0.05 vs. T), 11.7 \pm 1.2% (T+A), 13.3 \pm 0.8 (T+LN), 38.8 \pm 0.6 (T+LA+P, P>0.1 vs. T), respectively in the experimental groups. TNF induced ECV304 apoptosis was associated with increased intracellular superoxide production, and increased lipid peroxidation product malondialdehyde and cellular injury that was further exacerbated by L-arginine treatment. L-arginine exacerbated, while propofol as well as L-NNA and aminoguanidine attenuated TNF-induced apoptotic cell death. Furthermore, L-arginine cancelled the protective effects of propofol. In contrast, propofol and L-NNA conferred synergistic effects in reducing TNF-induced apoptosis.

Conclusion: Under pathological condition that is associated with increased TNF production, L-arginine supplementation may further exacerbate TNF cellular toxicity and unmask potential protective effects of propofol in attenuating TNF-induced vascular endothelial cell apoptosis.

SCA46

DIFFERENTIAL CARDIOVASCULAR RESPONSES OF nNOS IN MICE DURING ACUTE HEMODILUTIONAL ANEMIA AND SYSTEMIC HYPOXIA

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Neuronal nitric oxide synthase (nNOS) plays an important role in cardiovascular regulation. nNOS has been shown to regulate intracellular Ca²⁺ homeostasis and contractile function in the heart. Increase expression of nNOS has been shown during anemia and hypoxia. However, whether nNOS expression is protective or detrimental in regulating cardiac function during anemia and hypoxia has not been characterized. A mouse model of hemodilution was established to assess the role of nNOS during anemia.

We hypothesize that nNOS contributes to cardiovascular protective mechanisms during acute anemia but not in hypoxia. To study the effect of nNOS on survival, anesthetized nNOS knock-out (-/-) and C57BL/6 (wildtype control) mice were hemodiluted in steps or exposed to hypoxia (5% O₂) until mortality. To study the role of nNOS in cardiovascular function during anemia, cardiac output (CO) was measured under anesthesia using an Ultrasound Biomicroscope at baseline, immediately after hemodilution to a target hemoglobin (Hb) of 50-60 g/L, and up to 7 days of recovery. To assess the cardiovascular role of nNOS during hypoxia, CO was measured at baseline in normoxia (21% O₂ for 20 minutes), hypoxia (15% O₂ for 40 minutes), and normoxia (21% O₂). Two-dimensional left ventricular images were obtained for calculation of CO. Stroke volume (SV), heart rate (HR), fractional shortening (FS), left ventricular end-diastolic (LVEDV) and end-systolic volumes (LVESV) were also measured.

Anemic nNOS -/- mice died earlier and at higher Hb concentrations (36±5 g/L) than wildtype control mice (26±3 g/L, p<0.05). In contrast, hypoxic nNOS -/- mice survived longer than hypoxic

wildtype mice. CO and SV in nNOS -/- were blunted compared to wildtype control during anemia, whereas CO and SV were increased in nNOS -/- mice but not in wildtype during hypoxia. LVEDV was increased in anemic wildtype but not in nNOS -/-, however LVEDV was maintained in hypoxic nNOS -/- but not in wildtype. nNOS -/- mice had blunted CO response, and died earlier and at higher Hb concentrations during hemodilution, whereas during systemic hypoxia, nNOS -/- mice had increased CO and survived longer than wildtype mice. This observation suggests that nNOS is protective in acute anemia, but it is detrimental during systemic hypoxia. Anemic wildtype mice had increased CO, SV and LVEDV, indicating that increased preload and optimal diastolic function contributed. These responses were not seen in anemic nNOS -/- mice, suggesting that inadequate LV filling and/or diastolic dysfunction contributed to the absent CO response to anemia. In contrast, hypoxic nNOS -/- mice had increased CO, accompanied by increased SV and maintained LVEDV, indicating that preload is preserved. However, CO was not increased in hypoxic wildtype mice due to a reduction in LVEDV and SV, suggesting that hypoxia-induced vasodilation contributed to reduced CO response to hypoxia. Reduced systemic hypoxia-mediated vasodilatory responses in nNOS -/- mice during hypoxia may help increase venous return, which promotes CO. In summary, nNOS differentially regulates CO responses in anemia and hypoxia, and these nNOS-dependent cardiovascular responses contributed to the observed mortality in mice during anemia and hypoxia.

SCA47

EXPLORING THE INTERACTION OF REMOTE ISCHEMIC AND ANESTHETIC-INDUCED PRECONDITIONING PATHWAYS

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Objective: Both remote ischemic preconditioning and inhaled anesthetics produce myocardial protection against ischemia/reperfusion injury without prior ischemia of the target organ. This study examined the individual and combined effects of anesthetic preconditioning (APC) and remote ischemic preconditioning (RIPC) on myocardial protection against ischemia/reperfusion injury and the PI3k-Akt signaling pathway.

Methods: Four groups of rabbits (n=6) were examined; 1) APC treated (20-min inhalation of 2.5% sevoflurane and 20-min wash-out); 2) RIPC treated (four 5-min cycles of lower limb ischemia and reperfusion); 3) APC+RIPC combined treated and 4) Sham treated (Control). Hearts were excised within 5 min, and mounted on a flow-directed, recirculating Langendorff followed by 30-min global ischemia and 2-hrs reperfusion. Infarct size in heart slices (4-mm thick) was measured after tetrazolium staining and computerized planimetry. Akt signaling was assessed by levels of phosphorylated Akt (p-Akt) using Western blotting of protein samples from the mid-papillary portion of the left ventricle.

Results: APC and/or RIPC significantly reduced infarct size compared to Control (RIPC, $12.1 \pm 3.0\%$; APC $13.5 \pm 1.7\%$; APC+RIPC, $14.4 \pm 2.1\%$ vs. Control, $31.3 \pm 3.3\%$, all $P < 0.01$). However, no significant differences were found between each of the individual (RIPC, APC) or the combined (APC+RIPC) treatment. Compared to control, p-Akt was increased in RIPC hearts (1.5 fold), decreased in sevoflurane treated (0.67 fold) and was unchanged with combined APC and RIPC, but with no statistical significance.

Conclusion: While RIPC and APC are equipotent, the two preconditioning methods do not provide additive or synergistic cardioprotective effects. Furthermore, while our results suggest involvement of the PI3k-Akt pathway in the cardioprotective effects of RIPC, the decreased levels of p-Akt following sevoflurane-induced APC suggest that they differ in the details of their signaling pathways.

SCA48

IN-VITRO STUDY OF THE SMALL MOLECULE PROTEASE INHIBITOR CU-2010 – A NOVEL HEMOSTASIS MODULATOR WITH ANTIFIBRINOLYTIC AND ANTICOAGULATIVE PROPERTIES

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Background. The beneficial effect of non-specific protease inhibition on bleeding tendency and inflammatory response in cardiac surgery has been proven (1). However, the only approved drug for this indication, aprotinin, raised suspicion of severe side effects and its marketing is currently suspended. Moreover, aprotinin is of animal origin and has antigenic properties (2). Thus, alternative compounds are desirable. CU-2010 is a novel synthetic serine protease inhibitor with high affinity for plasmin, plasma kallikrein and factors Xa and XIa. CU-2010 is a 700 Da small molecule with rapid clearance after intravenous infusion elimination $t_{1/2}$ is approximately 20 minutes in rats and dogs. The aim of the present in vitro study was to evaluate the antifibrinolytic and anticoagulative effects of CU-2010 in established in vitro assays in comparison to aprotinin and tranexamic acid.

Methods. Antifibrinolytic activity in the blood of ten healthy volunteers was examined with a turbidometric method and with tissue factor activated thromboelastometry (Rotem®) and was compared to aprotinin and to tranexamic acid.

Results. CU-2010 displays high affinity for plasmin ($K_i = 2$ nM). This results in inhibition of tPA-induced fibrinolysis comparable to aprotinin and substantially more potent inhibition than tranexamic acid. As shown in the figure, CU-2010 and aprotinin largely suppress clot lysis at concentrations of 0.6 and 1 mM, respectively, while tranexamic acid requires concentrations between 3 and 10 mM for effective inhibition. In addition, CU-2010 shows moderate anticoagulative properties in human plasma (plasma kallikrein: $K_i \ll 1$ nM, FXa: $K_i = 51$ nM, F XIa: $K_i = 26$ nM) and whole blood that may be, in contrast to aprotinin, not restricted to the contact activation pathway.

Conclusion. These findings suggest that CU-2010 has similar inhibitory potency compared to aprotinin, yet accompanied by stronger anticoagulation. This anticoagulant profile may favorably mitigate thrombin generation during cardiac surgery. The beneficial effects of aprotinin exemplified a positive effect of non-specific serine protease inhibition during cardiac surgery. CU-2010 may serve as a model for small molecule attenuation of hemostatic activation, which is desirable during and after cardiac surgery.

References

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Figure 1. Inhibition of fibrinolytic activity as measured in Rotem®. The samples were activated with t-PA.

