

EXPERIMENTAL CIRCULATION

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Homocysteine induced cardiac dysfunction cannot be reversed with diet change in a rodent model of young versus aged animals

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Introduction: Anesthesiologists are managing patients with complex vascular diseases. Homocysteine (Hcy) is a sulfur containing amino acid produced in the metabolic pathway of Methionine (Met). Hyperhomocysteinemia (HHcy) has been identified as a nutritional risk factor for vascular disease, atrial fibrillation and thromboembolic events (1). Hcy levels increase with intake of Met-rich products ("Western diet"), sedentary lifestyle, and impaired renal function. Aging includes decrease in renal function and elevated risk for cardiovascular events. Measuring Hcy levels can be useful in patients who present with metabolic and vascular risk factors.

Objectives: To determine cardiac function in young and aged animals fed Hcy enriched diet and whether changing to a healthy diet at advanced age would lead to improved cardiac function.

Methods: C57BL6J male mice 12 weeks old were assigned to: (1) 12 weeks regular rodent food until 24 weeks old, (2) 12 weeks Hcy enriched diet until 24 weeks old, (3) 24 weeks regular rodent feed until 36 weeks old, (4) 12 weeks Hcy enriched diet with diet change to regular diet for 12 weeks until 36 weeks old (5 animals per group). 2D transthoracic echocardiography performed at 12 weeks, 24 weeks, and 36 weeks of age. Fractional shortening (FS), right ventricular inner diameter (RVID), left diastolic and systolic inner diameter (LVIDs, LVIDd) were assessed. ECG was monitored using an implanted telemetric device. Heart rate, PR interval, QRS interval and QT time were assessed over the last ten days of the study period.

Results: 24-week old animals fed Hcy diet had statistically significant enlarged LVIDd and RVID and significantly reduced FS. Diet change did not improve cardiac function. Telemetric ECG showed significant prolongation in QRS interval and QT time in animals fed Hcy.

Discussion: Changing from a Hcy enriched diet to a healthy diet after having consumed a poor diet for a major portion of the animals' life did not lead to any improvement of

cardiac function. Physiologic aging reduces cardiac function, however Hcy had a major influence on poor myocardial function in this model of aged animals.

Conclusion: Increased Hcy intake at young age leads to ventricular dysfunction. Hcy induced pathologic cardiac remodeling is unlikely to be reversed with simple diet change if Hcy was elevated for a significant lifespan. Aging related cardiac dysfunction seems to be accelerated with poor diet choices. Anesthesiologists might consider discussing perioperative complications in aged risk patients with a history of HHcy.

Reference

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Paper No: 479.00

Temporary Total Cardiopulmonary Support for Refractory Cardiogenic Shock (Two Levitronix CentriMag as BiVAD and ECMO inserted in the RVAD circuit)

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Introduction: At IKEM, we have used implantable ventricular assist devices (VAD) to treat end-stage heart failure since 2003. In 2009, we treated pandemic influenza (N1H1) patients with long-term extracorporeal membrane oxygenation (LT-ECMO) (1).

Objectives: A woman (28) underwent surgery for severe mitral and tricuspid regurgitation in another hospital (Day 0). On Day 6, progressive multiorgan failure and refractory shock state required a transfer to IKEM to implant total cardiopulmonary support combining BiVAD and LT-ECMO.

Methods: Day 7: Implanted two Levitronix CentriMag magnetically levitated pumps. LVAD (left atrium – ascending aorta; average flow 5.4 L/min) and RVAD (right atrium – pulmonary artery; flow 5.0 L/min). In the RVAD circuit we inserted oxygenator Quadrox PLS with highly resistant, extended-use polymethylpentene fibres. Previously inserted IABP and ECMO from femoral artery and vein were removed; applied continuous venovenous hemofiltration (CVVH). Day 10: Definitive suture of sternum. Day 14: Tracheostomy. The function of both heart ventricles and aeration of both lungs improved,

catecholamine support weaned; ECMO sweep gas flow reduced to 20%. Day 17: BiVAD end ECMO explanted. Day 18: Definitive sternal closure. Sedation discontinued; weaning from mechanical ventilatory support started in daytime, continued for patient's psychological comfort at night. Day 40: Spontaneous diuresis started. Day 47: CVVH terminated.

Results: On Day 53, the patient sent to original hospital for after-treatment w/ normal renal and liver function & good laboratory inflammatory parameters and satisfactory pre-release echocardiography. Positive subsequent recovery enabled patient's discharge, later to marry and resume teaching.

Discussion: Mortality of LCO after ECC is usually 80%. The inotropic support with IABP is the first step after primary failed weaning from ECC; the implantation of ECMO would be the next. LVAD implantation instead of ECMO was described with good results. The preoperative prediction of RV function after LVAD implantation is crucial for device selection and patient's outcome (2,3). RVAD placement avoids RV ballooning and irreversible cardiomyocyte stretching (6). Low mortality rate of 21% for application of ECMO for extended use in young adults with severe hypoxemia was documented (1).

Conclusion: Early recognition of patients rapidly progressing to refractory cardiac failure requires their immediate transfer to a hospital experienced in mechanical circulatory support. This cardiogenic shock patient suffered pulmonary, renal and hepatic failure and was risking ICU death. Even though the mechanical support was implanted relatively late (Day 7), saving the patient imbued our professional confidence in being able to apply the related complex interventions.

References

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Paper No: 657.00

Cellular mechanisms of cardiac dysfunction in endotoxemic mice

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Introduction: The pathology of cardiovascular dysfunction in sepsis and shock is incompletely understood. While nitric

oxide (NO, released by the inducible NO synthase, NOS2) is known to play a pivotal role, its downstream effectors are still unclear. In principle, NO acts by activating the enzyme soluble guanylate cyclase (sGC) to synthesize cGMP, and also has cGMP-independent effects, including oxidative modifications of proteins.

Objectives: Here we aimed to differentiate the roles of cGMP and oxidative stress in the dysregulation of cardiac calcium (Ca²⁺) handling induced by endotoxemic shock in mice.

Methods and Results: Cardiac myocytes were isolated from C57BL/6 mice at baseline and 12h after administration of lipopolysaccharide (LPS, 25 mcg/g, ip). LPS induced a decrease in externally paced cellular Ca²⁺ transients (measured with fura 2AM) and cell shortening to $78.25 \pm 0.04\%$ and $59.94 \pm 0.07\%$ of baseline, respectively ($n > 8$ mice). This was associated with a decrease in L-type Ca²⁺ channels (LTCC) current (to $76 \pm 4\%$ of baseline, measured by patch clamp) and expression (to $39 \pm 3\%$ of baseline, by immunoblotting, $n > 5$ mice for either). Sarcoplasmic reticulum Ca²⁺ pump (SERCA) activity was measured as the time constant of Ca²⁺ decay, and found to be decreased to $89 \pm 4\%$ of baseline after LPS. SERCA and phospholamban (PLB) expression levels, as well as PLB phosphorylation (at both Ser16 and Thr17 sites) were unchanged after LPS (immunoblotting, $n > 5$ mice). All the above deficits were found to be similar or more pronounced in mice deficient in the major isoform of sGC (sGC α 1-/-), indicating they are independent of cGMP formation. Moreover, for SERCA, LPS induced a decrease in the degree of biotinylated iodoacetamide (BIAM) labeling (to $61 \pm 6\%$ of baseline, $n > 6$ mice), indicating the presence of oxidative modifications, that have been shown previously to exert an inhibitory effect.

Conclusion: In mice, administration of LPS induces a decrease in LTCC expression and an allosteric inhibition of SERCA, that both contribute to cardiac dysfunction. Both effects are independent on cGMP and may be secondary to NO-induced oxidative modifications of LTCC and SERCA.

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Acute isovolemic anemia in sepsis : 6% hydroxyethyl starch versus 3% modified fluid gelatin

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Background and Goal of Study: Synthetic colloids are often used for fluid resuscitation during sepsis. Rheological properties of these colloids may affect tolerance to acute isovolemic anemia (AIA). This tolerance can be evaluated by the critical hemoglobin concentration (Hbcrit) defined as the hemoglobin (Hb) value below which O₂ consumption

becomes delivery dependent. The aim of our study was to compare the effects of 6% hydroxyethyl starch 130/0.4 (HES) and 3% modified fluid gelatin (GEL) on Hbcrit in an experimental model of sepsis induced in anesthetized sheep.

Materials and Methods: Following institutional animal research committee approval, 17 sheep were invasively monitored, anaesthetized with fentanyl (10 µg/kg) and sevoflurane (2% = 1MAC end-tidal), paralyzed with cisatracurium (0.15 mg/kg) and mechanically ventilated (FiO₂: 0.4). Sepsis was achieved by caecal ligation and perforation. Four hours after surgical preparation, animals were randomized to undergo progressive hemodilution using either HES (N=8) or GEL (N=9) as the substitution fluid (ratio 1:1). Each hemodilution step corresponds to a blood exchange of ± 500ml. For each sheep, Hbcrit was determined from a plot of O₂ consumption (VO₂: indirect calorimetry) versus Hb (Co-oximeter measurement) and from a plot of blood lactate (Lac) versus Hb using a last-sum-of-squares technique (1). Hbcrit values were compared between the two groups using a Mann-Whitney U test. Data are presented as median [interquartiles].

Results: Group HES Group GEL p Hbcrit (VO₂) (g/dl) 3.2 [2.5-4.0] 3.7 [3.3-4.4] NS Hbcrit (Lact) (g/dl) 3.3 [3.0-4.0] 3.3 [2.9-4.2] NS VO₂crit (ml/min) 271 [236-315] 329 [254-400] NS Lacrit (mMol/l) 0.9 [0.4-1.1] 1.6 [1.6-2.2] <0.01

Conclusion: In this experimental model of sepsis, the type of synthetic colloid used does not affect tolerance to AIA.

Reference

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Paper No: 918.00

Evaluation of the effect of three different inotropic support strategies in the normal and stunned newborn piglet heart on hemodynamics and myocardial metabolism

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Introduction: The myocardium of the newborn differs from the adult with regard to physiology, metabolism, and adrenoceptor density. A variety of inotropic strategies are used to treat low cardiac output in the newborn, all challenged by the low compliance of the myocardium and a high resting beta-adrenergic state.

Objectives: Aim of the present study was to evaluate the effect of three inotropic strategies on hemodynamics and metabolism in an in vivo neonatal piglet model with normal or stunned right ventricular myocardium.

Methods: Piglets were premedicated with midazolam and ketamine. Anesthesia was maintained with sevoflurane, and fentanyl infusion. Animals had pressure-volume catheters inserted in both the right and left ventricle. Microdialysis catheters were inserted in the left and right ventricle, and metabolites were measured in the dialysate. In half of the animals stunning of the right ventricle was induced by 10 cycles of 3 minutes of ischemia induced by a tourniquet around the right coronary artery, followed by 3 minutes of reperfusion. Animals followed a protocol with infusion for three hours with either: Dobutamin 8 µg/kg/min (DO); adrenaline 0.09 µg/kg/min and milrinone (loading dose of 50 µg/kg) 0.4 µg/kg/min (AM); dopamine 6 µg/kg/min and milrinone (loading dose of 50 µg/kg) 0.4 µg/kg/min (MD) or isotonic saline 2 ml/h.

Results: In the normal functioning hearts, heart rate increased significantly in all intervention groups, but no significant change was observed in CO. Contractility (dP/dt max) was significantly increased by AM (37%, p<0.05), and diastolic function (dP/dt min) was significantly improved by DO (32%, p<0.05) and AM (35% p<0.05). The lactate concentration increased significantly in both RV and LV microdialysate samples and plasma in the AM treated animals. In the animals with stunned right ventricle we found marked increase (197%, p<0.05) in lactate after ischemia-reperfusion. After 180 minutes blood lactate levels were significantly higher in the MA group, compared to all other groups. Preliminary results after 5 animals in each group stunning suggest DO treatment to have less effect on LV and RV contractility compared to other treatment groups. Results from microdialysis and pre and afterload independent measurements of contractility are pending analysis.

Conclusions: In the normal myocardium. The three inotropic strategies were comparable with respect to effect on hemodynamics, but MA had the most pronounced effect on contractility in terms of dP/dT. In the stunned myocardium, lactate remained high in the AM group after ischemia-reperfusion in contrast to the other intervention groups.

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Involvement of high mobility group box-1 in vascular and systemic responses to sepsis in rats

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Background: High mobility group box-1 (HMGB1) is one of the delayed-type mediators released during sepsis that enhance

inflammatory reactions in various organs. However, the role of HMGB1 in vascular responsiveness during sepsis is still unclear.

Objectives: To clarify the role of HMGB1 in vascular responsiveness and mortality.

Methods: Male SD rats (n=32, 200-250g in body weight) were used. CLP group rats (n=22) were subjected to cecal ligation and puncture under general anesthesia. Then, 4 mg/kg of anti HMGB1 antibody (AB group, n=11) or the same volume of normal saline (NS group, n=11) was injected through the tail vein immediately and 4 hrs after the surgery. A group of sham-operated rats (n=10) underwent laparotomy, and the cecum was manipulated but neither ligated nor punctured. The thoracic aorta was removed 12 hrs after the operation and phenylephrine (PE)-induced contraction was determined as the relative response to 40 mM KCl. Expression of HMGB1 was examined immunohistochemically or by Western blot analysis. In some rats (5 from each group), a transmitter was implanted in the abdomen to monitor heart rate and body temperature, and the femoral artery was catheterized to monitor arterial blood pressure for 16 hrs.

Results: In the first series of PE-induced contraction, the maximum responses were attenuated in the NS and AB groups compared to the sham group. In the second series, the responses were comparable to the first ones in the sham and AB groups, but were lower than the first ones in the NS group. In the NS group, HMGB1 was strongly expressed in the vascular endothelium compared to the sham group. Western blot analysis showed that HMGB1 expression was comparable in the sham and AB groups, but increased in the NS group in a time-dependent fashion. Arterial blood pressure was comparable in the AB and NS groups, but it was significantly decreased compared to that in the sham group. Heart rate was also comparable in the AB and NS groups, but it was significantly increased compared to that in the sham group. Body temperature was comparable in the three groups.

Conclusions: These findings suggest that HMGB1 significantly suppress the vascular contractile response in sepsis. The eight mg/kg dose of anti HMGB1 antibody was not sufficient to suppress the systemic inflammatory responses induced by cecal ligation and puncture.