Christopher et al – Methods Supplement

Rat model of low flow

The rat model of low blood flow was adapted from a previously published method (Karim et al. 2004; Chen et al. 2006). The surgical operation, pre- and post-operative palliative procedures were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University. Adult male Sprague Dawley rats were anesthetized using 1 mL/kg of an intraperitoneal injection mixture (3:3:2) of ketamine (100 mg/mL), xylazine (20 mg/mL), and acepromazine (10 mg/mL). The animals were intubated using a pediatric laryngoscope and a 14-gauge angiocatheter (Becton Dickinson, Franklin Lanes, NJ). The rats were ventilated continuously on room air supplemented with oxygen using a mechanical ventilator (Model 683, Harvard Apparatus, Holliston, MA). The chest was open via a left lateral thoracotomy at the fifth intercostal space. Once the heart was exposed and the pericardium removed, an apical suture was used to exteriorize the heart. The left anterior descending (LAD) coronary artery was visualized at the base of the ventricle, just below the left atrium. A 6-0 prolene suture (Ethicon, Piscataway, NJ) was looped through the ventricle wall, deep to the LAD. The ends of the suture were secured with needle holders, and a 24-gauge angiocatheter was placed on the anterior surface of the heart, parallel to the LAD. The suture was tied tightly around the angiocatheter, restricting flow to the LAD. If noticeable blanching occurred in the ventricle, the suture was deemed to be properly restricting the LAD. Once the suture was tied tightly, the angiocatheter was gently slid from within the suture. At this time, blood flow returned to the LAD and blanching disappeared, returning the heart to its normal color. The chest was closed in three layers using 3-0 vicryl suture, and the rats were removed from ventilation and placed in a recovery chamber with a warming light. Sham operated animals underwent the exact same procedure, except that the LAD suture was not tightened nor left in the heart. Rats in both groups were then euthanized three days post surgery and the hearts were removed for study. Approximately 90% of the sham operated animals survived surgery, whereas 60-70% of the experimental animals survived surgery. Rat hearts from both groups were devoid of gross morphological

changes, and tissue sections from the rat hearts stained by hematoxylin and eosin did not indicate myocardial infarction in either group of rats (data not shown).

Echocardiography

Both sham operated and experimental animals underwent echocardiography to assess left ventricular function three days after surgery. As previously described (Morgan et al. 2004), a small animal cardiac ultrasound machine (Sequoia C256 Echocardiography System, Siemens Medical) was used to collect 2-D digital loop images from animals that were consciously sedated using vaporized isoflurane (2% volume mixture). Parasternal long and short axis views were taken with a 15 MHz linear array transducer. At the end of the study, an operator blinded to the animal groups analyzed the accumulated data using software resident on the ultrasound system. Left ventricular end systolic (LVSd) and left ventricular end diastolic diameters (LVDd) were measured and fractional shortening was calculated as [(LVDd - LVSd)/LVDd] x 100%. Measurements were taken at rest, and with sequential dobutamine dosing to 1.5 μg/g rat weight (Low Dose) and 4.5 μg/g rat weight (High Dose). Results are presented as average ± standard deviation and differences between groups were deemed statistically significant if $P < 0.05$.

Mass Spectrometry

Troponin I samples were analyzed by mass spectrometry at two independent facilities (Mayo Medical School Mass Spectrometry Facility and 21st Century Biochemicals). Proteins were digested by trypsin, or acetylated prior to chymotrypsin digestion and analyzed by LC-MS or tandem MS. For tandem MS, the digest peptide mixture was loaded onto a 250 nL OPTI-PAK trap custom packed with Michrom Magic C8 solid phase. Chromatography was performed using 0.2% formic acid in both the A solvent (98% H_2O : 2% acetonitrile) and B solvent (80% acetonitrile : 10% isopropanol : 10% water), and a 5% B to 45% B gradient over 30 min at 400 nL/min through a Michrom packed tip capillary (Magic C18 75μm x 150mm) column. The ThermoFinnigan LTQ Orbitrap Hybrid mass spectrometer experiment was set to perform an FT full scan from

375 - 1600 m/z with resolution set at 60,000 (at 400m/z), followed by linear ion trap MS/MS scans on the top five ions. For LC-MS, chromatography was performed using 150 μm column packed in-house using Michrom C-18 reversed-phase packing material. Separation was performed using an LCPacking Ultimate micropump with 98:2 H_2O : acetonitrile with 0.1% formic acid and 0.001% trifluoroacetic acid as buffer A and 80:10:10 acetonitrile : H_2O : isopropanol with 0.1% formic acid and 0.001% trifluoroacetic acid as buffer B. The column was interfaced to an MDS/Sciex QStar XL mass spectrometer.

References

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