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Meeting Abstracts Ischemic conditioning and targeting reperfusion injury: a 30 year voyage of discovery, *May 12th – 13th, 2016, Barcelona*

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Effect of lower limb remote ischemic conditioning on infarct size in patients with anterior ST-elevation myocardial infarction

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Background: Previous studies indicate that remote ischemic conditioning performed before percutaneous coronary intervention (PCI) reduces infarct size in patients with ST-elevation myocardial infarction (STEMI). It remains unclear whether remote conditioning affords protection when performed in adjunct to primary PCI. The aim of the study was to test whether remote ischemic per-postconditioning (RIperpostC) initiated after admission to the catheterization laboratory attenuates myocardial infarct size in patients with anterior STEMI.

Methods: Ninety-three patients with anterior STEMI were randomized to RIperpostC or sham procedure as adjunct to primary PCI. RIperpostC was started on arrival in the catheterization laboratory by 5 min cycles of inflation and deflation of a blood pressure cuff around the left thigh and continued throughout the PCI procedure. Infarct size and myocardium at risk were determined by cardiac magnetic resonance after four to seven days. The primary outcome was myocardial salvage index (MSI).

Results: There was no significant difference in MSI between the RIperpostC and control group (median 48.5% and interquartile range 30.9%-60.8% vs. 49.2% (42.1%-58.8%)). Neither did absolute infarct size in relation to left ventricular myocardial volume differ significantly (RIperpostC 20.6% (14.1%-31.7%) vs. control 17.9% (13.4%-25.0%)). The RIperpostC group had larger myocardial area at risk than the control group (43.1% (35.4%-49.7%) vs. 37.0% (30.8%-44.1%) of the left ventricle, $p = 0.03$). Peak value and area under the curve for troponin T did not differ significantly between the study groups.

Conclusions: RIperpostC initiated after admission to the catheterization laboratory in patients with anterior STEMI did not confer protection against reperfusion injury.

2

Cardioprotective effect of Ranolazine in the process of ischemia-reperfusion in adult rat cardiomyocytes

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Background: Ranolazine is used as a complementary treatment for angina in patients. Ranolazine inhibits sodium voltage-dependent channels, and is supposed to prevent sodium and calcium overload during ischemia and reperfusion (I/R). Here we characterized the effect of Ranolazine on calcium homeostasis in isolated adult rat cardiomyocytes subjected to simulated I/R.

Methods: Transients of intracellular calcium concentration ($[Ca^{2+}]_i$) changes were evaluated using microfluorimetry with fluorescent indicator Fura-2, and by confocal microscopy with the indicator Fluo-3 in isolated rat cardiac myocytes.

Results: We found that cells subjected to I/R showed increased diastolic calcium concentration, while the amplitude of $[Ca^{2+}]_i$ was attenuated. The addition of ranolazine only during ischemia improved significantly the $[Ca^{2+}]_i$ handling, preventing $[Ca^{2+}]_i$ overload, decreasing the diastolic calcium and keeping the amplitude of the intracellular calcium transient, which is reflected by a successful recovery in the process of excitation-contraction coupling during reperfusion. However, these effects weren't observed when ranolazine was added only in reperfusion, nor during ischemia and reperfusion.

Conclusions: Ranolazine shows beneficial effects in cardiomyocytes exposed to ischemia/reperfusion, only when applied during ischemia. Ranolazine decreases Ca^{2+} overload and improves the calcium homeostasis.

3

Changes in cardiac survival signaling during postnatal development: implications for cardioprotection

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Background: Previous work from Bristol has shown that resistance to ischemia/reperfusion (I/R) follows a bell-shaped profile during postnatal development, with 2 week old rats displaying the greatest degree of resistance. The mechanisms underlying these differences are not currently known. The aim of this study was to monitor the changes in survival signaling proteins during postnatal development, and to determine whether these changes correlate with the cardiac vulnerability profile previously discovered in response to I/R.

Methods: Cardiac protein extracts from 14 day ($n = 3$), 28 day ($n = 3$) and adult ($n = 4$) rats were processed using Tandem Mass Tag (TMT) proteomic analysis. Proteins involved in survival signaling or apoptotic processes were then identified, and statistical analysis (One-way ANOVA) was performed to compare protein expression between the three age groups. Western blotting was then carried out (expressed as a ratio to GAPDH) to validate the proteomic data.

Results: 26 survival signaling proteins were identified from the proteomic output; 23 displayed a statistically significant decrease with age, including Caveolin-1 & 3, and Heat shock protein 90 (HSP90). A significant increase with age was seen with Apoptosis-inducing factor 1 (AIF1). These patterns of protein expression were successfully validated using Western Blotting.

Conclusions: Our data indicate a strong correlation between pro-survival signaling protein expression and vulnerability to I/R during postnatal development. However, these findings are correlative, and do not prove a cause and effect relationship. Further work will be required to explore this relationship between survival signaling proteins, postnatal development, and I/R.

4

Rho kinase inhibition protects from ischemia-reperfusion injury via a mechanism related to nitric oxide synthase and downregulation of arginase in diabetes

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Background: Activated RhoA/Rho associated kinase (ROCK) and arginase are implicated in vascular complication in diabetes. The present study investigated the cardioprotective effect of ROCK inhibition and its combination with remote ischemic perconditioning (RIPerc) in type 1 diabetes.

Methods: Anesthetized non-diabetic and streptozotocin-induced type 1 diabetic SpragueDawley rats were subjected to 30 min regional myocardial ischemia and 2 h reperfusion (IR) and allocated to (1) controls with no intervention during IR; (2) ROCK inhibition with hydroxyfasudil; (3) RIPerc (femoral artery occlusion for 15 min during the last 15 min of the myocardial ischemia); (4) ROCK inhibition + RIPerc, (5) NO synthase (NOS) inhibition by L-NMMA alone and (6) NOS inhibition combined with ROCK inhibition. Myocardial ROCK and arginase activity, arginase expression as well as infarct size (IS) were determined.

Results: Arginase activity and arginase 2 protein expression as well as ROCK activity were increased in type 1 diabetes ($P < 0.05$). While RIPerc failed to induce cardioprotection in rats with diabetes, ROCK inhibition alone and in combination with RIPerc significantly reduced IS and arginase activity in both nondiabetic and diabetic rats by comparable magnitudes ($P < 0.05$). The cardioprotective effect and the downregulation of arginase activity by ROCK inhibition in diabetes were abolished by NOS inhibition.

Conclusions: ROCK inhibition induces marked cardioprotective effects in rats with type 1 diabetes. The cardioprotective effect of ROCK inhibition in diabetes is mediated by a NOS-dependent signaling pathway associated with a decrease in arginase activity. This finding may be a potential therapeutic strategy to protect the diabetic heart against IR injury.

5

Attenuation of myocardial and vascular arginase activity by vagal nerve stimulation during cardiac ischemia and reperfusion involves alpha-7 nicotinic receptor

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Background: Vagal nerve stimulation (VNS) protects from myocardial and vascular injury following cardiac ischemia/reperfusion (IR) via a mechanism involving activation of alpha-7 nicotinic acetylcholine receptors (α7 nAChRs) and reduced inflammation. Arginase is involved in development of IR injury driven by inflammatory mediators. The aim of the study was to clarify whether VNS attenuates IR-induced arginase upregulation in the myocardium and aorta via a mechanism acting on α7 nAChRs.

Methods: Anaesthetized Sprague-Dawley rats subjected to 30 min left coronary artery ligation followed by 2 h reperfusion were allocated to: (1) sham ($n = 5$); (2) control IR ($n = 10$); (3) VNS ($n = 13$, VNS throughout IR) and (4) methyllycaconitine ($n = 7$, MLA; 10 mg/kg ip, an α7 nAChRs blocker) + VNS group. Infarct size and arginase activity were determined.

Results: VNS reduced infarct size compared to control IR (41 $\pm$ 3% vs. 66 $\pm$ 3% of risk area, $P < 0.001$). Myocardial IR increased arginase activity 1.6-fold ($P < 0.05$ vs. sham) in the myocardium at risk and 3.1-fold ($P < 0.001$ vs. sham) in aorta. VNS attenuated the increase in arginase activity compared to control IR both in the myocardium and aorta ($P < 0.05$). The administration of MLA partially abolished the cardioprotective effect of VNS ($P < 0.05$) and completely abrogated the effect of VNS on arginase activity, without affecting heart rate.

Conclusions: VNS reduced infarct size and reversed the upregulation of arginase both in the myocardium and aorta via a mechanism depending on α7 nAChRs activation. This finding may represent a novel cardiovascular protective effect of VNS mediated via attenuated arginase activity.

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Kinetic oscillation stimulation of the nose: a novel treatment for myocardial ischemia-reperfusion injury

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