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Minocycline, a Second Generation Tetracycline with Proven Safety, Protects Cardiac Myocytes against Both Ishaemia and Reperfusion Injury

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Background Minocycline (MCN) is a second-generation tetracycline that is used in humans for the treatment of acne and urethritis as well as of severe chronic inflammatory diseases. In previous studies carried out in animal models of cerebral ischaemia, MCN provided a significant neuroprotection by decreasing the expression of some caspases and pro-inflammatory agents. This study was aimed to evaluate whether MCN could protect cardiomyocytes (CM) against hypoxia (H) and reoxygenation (R) injury. Materials and Methods CM isolated from hearts of neonatal and adult rats were exposed to H by incubation in a hypoxic chamber, followed, in some experiments, by (R). MCN was added to CM 0, 1, 2, 4 and 24 hours prior to H or at R, using 3 different concentrations (10, 1 and 0.1 mM). Necrotic and apoptotic cell death (N- and ACD) were assessed by fluorescent microscopy (PI staining after cell fixation to measure NCD followed by TUNEL and PI staining after permeabilization to measure ACD) and FACS analysis (Annexin V and PI staining). The expression of caspase 8 and 9 (initiator caspases) and caspase 3 and 7 (effector caspases) was evaluated by Western Blotting (WB) and immunocytochemical staining (IS). Results MCN reduced the magnitude of N- and ACD when given to CM both prior to H and at R. The cardioprotective effect peaked when MCN was administered 2 hours before H. In neonatal CM, for example, NCD decreased from 35.2% to 24.5% and ACD from 28.3% to 18.1% ($p<0.001$). Cardioprotection was still observed when MCN was added at the onset of R. The extent of N- (38.1%) and ACD (29.5%) in adult CM diminished e.g. to 31.7 and 22.4% respectively ($p<0.05$). By WB, MCN given both before H and R reduced the expression of all examined caspases and the cleavage into their active peptides, as confirmed by IS with antibodies recognizing the cleaved active form of caspase 3, 7, 8 and 9. Conclusions Our data suggest that MCN effectively improves tolerance to ischaemia in CM pretreated both before H and at R. Consistent with this finding is the ability of MCN of reducing expression and activation of caspases. These results, together with the proven safety of MCN, seem to suggest that MCN might be successfully employed in humans as a novel cardioprotective agent in different ischaemic conditions.

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Elevated Glucose Concentration Exaggerates the Inhibitory Effects of Balloon Angioplasty on Arterial Vasoreactivity

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Background: Intravascular interventions in diabetics is associated with worse outcomes than non-diabetics.It is well known that vasomotor relaxation is inhibited by arterial injury. We designed this experiment to investigate the effect of elevated glucose concentration on the vasomotor response following balloon angioplasty (BA). Methods: Carotid arteries from 22 New Zealand White rabbits were isolated then perfused in a dual organ chamber with physiologic buffered solution (PBS) using varying glucose (G) concentrations: 100 (G100, n = 20), 250 (G250, n = 17) or 500 (G500, n = 3) mg/dl . Four arteries were perfused with PBS at 100 mg/dl glucose plus 150 mg/dl mannitol (M) (G100/M150) to achieve equal osmolarity to G250. Baseline vasoreactivity was determined using norepinephrine (NE, 2 × 10-6 M) preconstriction. Then, pharmacologic challenge was performed with acetylcholine (Ach, 2 × 10-5 M) and sodium nitroprusside (SN, 2 × 10-5 M). BA was performed with a 2.5 by 15 mm balloon catheter followed by pharmacologic re-challenge. Vessel diameter was measured using a computer planimetry technique. The ratio of percent vasomotor relaxation after BA to before BA was used to compare changes in vasomotor response. Results: BA significantly reduced carotid vasoreactivity at all glucose concentrations, however with G250 and G500 there was further significant reduction when compared to G100 or to G100/M150 ($p<0.05$) (table 1) Conclusion: BA at high glucose concentration is associated with more reduction in arterial vasodilation when compared to BA at normal glucose level. The mechanism seems to be related to a non-osmotic effect of higher glucose on the injured vascular wall.

Ratio of Percent Arterial Vasodilation Before and After BA

Concentration(mg/dl)	(Ach-NE)/NE	(SN-NE)/NE
G100	$0.43 \pm 0.35^{*}$	$0.49 \pm 0.12^{*}$
G100/M150	$0.47 \pm 0.12^{*}$	$0.49 \pm 0.15^{*}$
G250	$0.27 \pm 0.14^{**}$	$0.30 \pm 0.17^{**}$
G500	$0.31 \pm 0.12^{**}$	$0.31 \pm 0.14^{**}$

** $p<0.05$ compared to *

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Intimal Hyperplasia in Arterialized Venous Grafts in a Canine Model

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Previous studies from this laboratory focused on myocardial revascularization by gene therapy with VEGF to treat non-reconstructable ischemic heart disease. However conventional revascularization remains the mainstay of treatment. Each year over 600,000 coronary artery bypass grafts (CABG) are carried out in the US alone. Of these, up to 15% fail within 1 year after surgery, and up to 40% fail by 10 years. Although the characteristics of failing grafts are well known, the underlying molecular mechanisms are only poorly understood. To gain insights into these mechanisms that would help improve graft life, we have carried out studies to examine global patterns of gene expression in venous grafts using DNA microarrays. Arterialization was studied in various venous grafts in dogs. In this model, venous grafts are placed between the aortic arch and the circumflex artery via a lateral thoracoctomy, and in the same animal venous interposition grafts are placed into the carotid artery. These parallel grafts provide the opportunity to compare the arterialization process in different hemodynamic settings. Animals were maintained for 3-, 10-, and 30-days post operatively. The grafts were harvested for histological analysis and RNA extraction. Gross examination at sacrifice revealed thickened vessel walls of the venous grafts at the 10- and 30-day time periods with no obvious hypertrophy in the 3-day groups. Histophathologic and DNA microarray analyses of gene expression are in progress. By comparing the pattern of gene expression in normal and failing grafts we hope to identify new targets for gene therapy (and possibly new drugs) to improve surgical outcomes, and to relieve patients of the need for repeat surgery.

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Na^+-H^+ Exchanger Subtype 1 (NHE_1) Inhibition Limits Ischemia and Reperfusion-Induced Coronary Vascular Dysfunction

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Myocardial ischemia and reperfusion cause vascular dysfunction that is frequently termed "stunning". We have shown previously in rats that NHE_1 inhibition limits coronary vascular dysfunction after two repetitive left coronary artery (LCA) occlusions, followed by 15-min of reperfusion. The purpose of the present study was to determine whether NHE_1 inhibition using Cariporide (CAR) lessens ischemia and reperfusion-induced dysfunction of coronary resistance vessels after a longer (60 min) period of reperfusion. Male Sprague-Dawley rats were anesthetized and instrumented to measure regional and global myocardial function, and to reversibly occlude the LCA. After pretreatment with vehicle (saline; n=4) or CAR (7 mg/kg iv bolus, 0.07 mg/kg/min constant infusion; n=4), animals completed 2 x 10 min LCA occlusions separated by 5-min of reperfusion, followed by 60-min of reperfusion. After excising the heart, coronary resistance vessels (~110 µm, internal diameter) were isolated, mounted in a myograph, and endothelium-dependent (acetylcholine, ACh) and independent (sodium nitroprusside, SNP) dilation, and endothelin-1 (ET-1) -induced constriction were assessed. Responses from 2-4 arterial segments were averaged per animal. ACh-induced relaxation ($3x10^{-6}$ M, 10^{-5} M, $3x10^{-5}$ M) in vessels obtained after 60-min of reperfusion was greater ($p<0.05$) in CAR-treated (47±10, 54±8, 60±6%) vs vehicle-treated rats (29±4, 34±6, 36±6%). SNP-evoked relaxation and ET-1 -induced contractile responses were similar between groups. These preliminary data, combined with our earlier findings, indicate that NHE_1 inhibition lessens ischemia-induced coronary vascular dysfunction after short (i.e., 15-min) and long (i.e., 60-min) periods of reperfusion.