Effects of High-Dose Cariporide in a Long-Term Ischemia/Reperfusion Model

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We hypothesized that a high-dose (1.5 mg/kg, every 8 hours for 3 days) of the Na/H exchange (NHE) inhibitor cariporide could improve recovery following a reperfused myocardial infarction. Using a 2-hour occlusion/10-day reperfusion canine MRI/S model we assessed function, metabolism, and myocardial necrosis. Cariporide reduced pH during ischemia and at 3 and 10 days post-reperfusion ($p<0.05$). Ejection fraction (EF) improved immediately upon reperfusion (% of baseline; 63.5%±3.5% controls, 90.5%±7.2% cariporide) and at day-3 ($p < 0.05$). At day-10 infarct ratios (controls 57.6%±7.9%, cariporide 58.4%±5.5%) and EF were similar in both groups. In a clinically relevant model, cariporide provides only short-term protection following a reperfused ischemic insult.

Introduction

Although reperfusion is the therapy of choice for patients with acute ST segment elevation myocardial infarction (AMI), it alone does not optimize the salvage of ischemic myocytes. Various pharmaceuticals have been developed with the aim of reducing reperfusion injury, one such drug is cariporide, a NHE inhibitor. This study assessed the potential benefits of cariporide in a clinically relevant model of ischemia/reperfusion (I/R).

Methods

22 female beagles underwent a 2-hour occlusion followed by 10 days of reperfusion within this protocol; 10 were randomly selected as controls, 12 received cariporide (1.5 mg/kg) just prior to occlusion, immediately upon reperfusion, and every 8 hours for the following 2 days. Cine MRI was interleaved with ^{31}P MRS to assess both the functional and metabolic affects of cariporide. Radioactively labeled microspheres were injected for coronary blood flow evaluation. Contrast-enhanced MRI was also performed to determine absolute infarct volumes (IV).

A left thoracotomy was performed, the left anterior descending coronary artery was dissected free, and a ligature was placed around the artery. The left atrial appendage and a femoral artery were catheterized and the dog was transported to the Siemens 1.5T MR suite. Figure 1 depicts the protocol. Cine MR images were acquired in a rigid ^{1}H rf coil with an ECG-gated, breath-hold, segmented 2D-FLASH sequence (TR/TE 60/4.8ms, α 20°, Thickness 6mm). 5-6 short axis slices were imaged through the entire heart with 8-10 cardiac phases depending on heart rate. Multivoxel (16x16) 2D-Chemical Shift Imaging (CSI) was acquired on a dual-tuned ^{1}H/^{31}P surface coil (TR 500ms, α ~50°, FOV 320mm, BW ±2kHz). Just prior to sacrifice a bolus and constant infusion of Gd-DTPA was injected, followed by *ex vivo* imaging with a 3D FLASH sequence (TR/TE 22/10ms, FOV 80-100mm, Thickness 1mm).

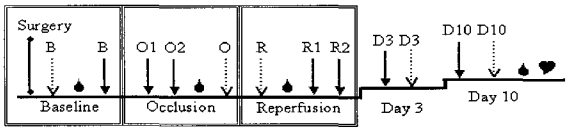

Figure 1: Experimental protocol used for all dogs. Solid arrows are ^{31}P CSI, hashed arrows cine MR, ♦ blood flow measurements, and ♥ heart excision and imaging. B, O, O1, O2, R, R1, R2, D3, and D10 correspond to baseline, occlusion, occlusion 1 and 2, reperfusion, reperfusion 1 and 2, day 3 and 10 respectively.

Cine MR images were analyzed with Siemens' ARGUS software package. End diastole (ED) and end systole (ES) endocardial contours were drawn for each slice and EF was then determined from the difference in blood volumes normalized by the ED volume. ^{31}P CSI raw data were filtered and zero filled (2048 points) by Siemens' software. Using FITMAN and prior knowledge all spectra were fitted in the time domain (1). The chemical shift between the phosphocreatine (PCr) and inorganic phosphate (Pi) peaks was used to determine intracellular pH within the healthy and damaged myocardium. Contrast-enhanced images were volume rendered using AnalyzeAVW to determine IV's.

Results

Normalized EF data presented in Figure 2 demonstrate the improved early functional recovery in the cariporide group (n=11) as compared to the controls (n=10).

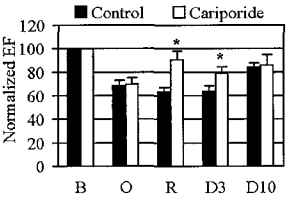

Figure 2: Mean baseline normalized EF data (± SEM error bars) to account for animal variability. Between group significance *, $p<0.05$.

Intracellular pH was significantly lower ($p<0.05$) in the cariporide group over the entire 10-days and specifically at O2, D3 and D10. There were no PCr/adenosine triphosphate ratio differences between the groups at any time-point. IV's were similar in both groups (Table 1). The regions of low flow (<0.3ml/min/g during occlusion, as determined by the radioactively labeled microsphere injections) were comparable, indicating the consistency of our model. Normalizing the IV by the low flow regions resulted in infarct ratios (Table 1).

	Infarct Volume (% LV)	Low Flow Region (% LV)	Infarct Ratio (%)
Control (n=9)	15.7 (3.0)	26.2 (1.9)	57.6 (7.9)
Cariporide (n=12)	15.8 (1.8)	27.3 (2.0)	58.4 (5.5)

Table 1: Mean (±SEM) infarct volumes, low blood flow regions, and infarct ratios for both groups (NS); LV, left ventricle.

Discussion

This study demonstrates the long-term effects of cariporide in the setting of I/R. NHE inhibition caused the anticipated slowing of recovery of intracellular pH, as well as an immediate improvement in cardiac function. However, these functional benefits were not long lasting, despite the sustained reduction in pH. By day-10 there were no significant differences in function or viability between the cariporide and control groups. Many laboratories have reported the benefits of NHE inhibition in the setting of short-term I/R (2). However, in a long-term setting such as this study, as well as the two major clinical trials, the benefits on myocardial or patient viability have not been shown (3). It is well known that the complete extent of myocardial necrosis following I/R is not manifest until at least 72-hours; however the majority of laboratory studies do not last more than 24-hours (2, 4). Previously we have been able to reduce myocardial necrosis with various pharmaceutical interventions, including superoxide dismutase, using our long-term 2-hour occlusion/reperfusion canine model (1). In the setting of the current study, cariporide is blocking the NHE and providing short-term cardioprotection; however, during this lengthy period of I/R the production of oxygen free radicals, neutrophils, and macrophages, as well as the activation of the complement cascade, limit the benefits of this therapy on reducing reperfusion injury (4, 5). Considering that most patients present to the hospital with an AMI >2.1 hours following the onset of symptoms, pharmaceutical interventions need to offer protection against these other mediators (6). Extending the study of reperfusion to allow for the complete evolution of myocardial necrosis and apoptosis as seen in our 2-hour occlusion/10-day reperfusion canine model offers a much more clinically relevant assessment of the potential cardioprotection garnered by these pharmaceutical interventions.

Acknowledgements

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