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Cyclo-oxygenase-2 (COX-2) inhibition reduces apoptosis in acute myocardial infarction

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Cyclo-oxygenase-2 (COX-2) is, the enzyme catalyzing the conversion of arachidonic acid to prostaglandin H2, is not expressed in healthy myocardium and but its expression is induced in response to stress, such as ischemia.[1] Although COX-2 exerts beneficial effects in delayed ischemic preconditioning,[1] COX-2 inhibition appeared to be beneficial in animal models of AMI in terms of reduced infarct size, improved hemodynamic performance, and improved cardiac remodeling.[2,3] The mechanisms underlying such benefits in AMI are not yet completely clear. COX-2 expression in subjects with AMI was associated with significantly higher rates of cardiomyocyte apoptosis which may explain progressive cell loss and adverse remodeling.[4] Interestingly COX-2 expression was found in the very same cell undergoing DNA fragmentation[4] and death raising the issue whether COX-2 itself could be mediating the negative effects of ischemia by promoting apoptosis.

Aim of this study was to assess whether COX-2 inhibition could prevent cardiomyocytes from dying by apoptosis in an experimental model of AMI in mice in which surgical coronary ligation was performed.

Methods

Twenty male mice (5 weeks of age, 20 g of weight) underwent coronary ligation. The surgical procedures were performed on day 1 by two skilled operators (FL and AG) as previously extensively described.[5] Briefly, C57BL/6 male mice under anesthesia (ketamine 100 mg/Kg and acepromazine 1 mg/Kg) underwent surgical opening of the chest and ligation of the proximal left coronary artery. All animals survived surgery and were allowed to recover. Starting on day 2 (24 h after surgery), 9 mice were treated with a soluble COX-2 inhibitor, parecoxib (0.75 mg/Kg intraperitoneal) daily for 5 days and the remaining 10 received saline intraperitoneal injection. On day 7, the animals were sacrificed under anesthesia using cadmium chloride (Figure 1). Transverse sections of the median third of the left ventricle were taken and immediately fixed in paraformaldehyde as previously specified.[4,5] Infarct size was quantified as a percentage of the whole circumference. Specific staining for COX-2 was assessed using a primary antibody (goat polyclonal sc-1745, Santa Cruz Biotechnology, CA, US) at the dilution of 1:100. Apoptosis was defined by co-staining for TUNEL (DNA fragmentation—Oncor, Gaithersburg, MD), and activated caspase-3 (cleaved caspase-3 [Asp 175] antibody from Cell Signaling Technology, Beverly, MA, US; dilution 1:50). The detailed protocol was published elsewhere.[4] The peri-infarct area was defined as the zone bordering the infarct where viable myocardium was prevalent and reparative fibrosis was only marginal.[6] The apoptotic rate (AR) was expressed as the number of apoptotic cardiomyocytes on all cardiomyocytes per field. AR in the peri-infarct regions was calculated on 5 random fields, which virtually cover the entire peri-infarct area. The allocation to different treatments was randomized, and the pathologist was unaware of the treatment.

Statistical analysis was performed using the SPSS 11.0 package for Windows. Continuous variables are expressed as mean and standard error (standard deviation/sqroot[N]) and *T* test for unpaired data was used to compare groups. Discrete variables are expressed as percentage and the

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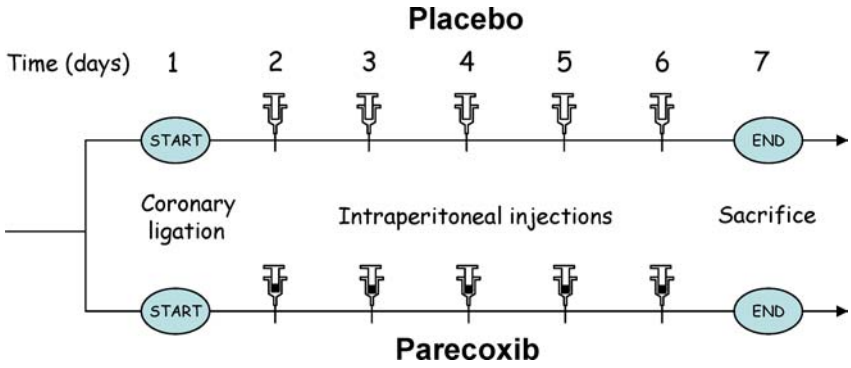
Figure 1. Design of the study. Twenty-four hours after surgery, rats were divided into 2 groups: one group received intraperitoneal parecoxib and one group normal saline. On day 7, all animals were sacrificed for histology.



Chi-square test or Fisher s exact test were used to compare groups.

Results

Of the 20 animals undergoing surgery, 1 animal died within the first 24 h (5%), 9 mice were treated with parecoxib and 10 with normal saline starting on day 2. Body weight at baseline was similar in the 2 groups.

Survival to day 7 was not different in the 2 groups (44% vs 50%, P = NS). Most of the deaths occurred between 48 and 72 h in both groups, due to infarct expansion, free wall rupture or presumed ventricular arrhythmias. Ten animals survived to day 7 and were sacrifed: 5 in the parecoxib treated group and 5 in the normal saline group. Infarct size was similar in the 2 groups (58% $\pm$ 8% vs 65% $\pm$ 6%, P = 0.63). Apoptosis in the peri-infarct region was significantly lower in the parecoxib treated animals (2.1% $\pm$ 0.3% vs 4.3% $\pm$ 0.4%, P = 0.007), showing a >50% reduction in the apoptotic rates. When analysis was extended to all available cases including those animals that died prior to day 7 but had received at least 1 dose of parecoxib or saline, treatment with parecoxib was still associated with significantly lower apoptotic rates (2.6% $\pm$ 0.4% vs 4.7% $\pm$ 0.6%, P = 0.009). The 4 mice who received >1 but <5 parecoxib doses had intermediate AR (3.2% $\pm$ 0.5%).

COX-2 expression was found in all cases in the peri-infarct area and in none of remote areas (P < 0.001) (Figure 2A). Of note virtually all apoptotic cardiomyocytes expressed COX-2.

Discussion

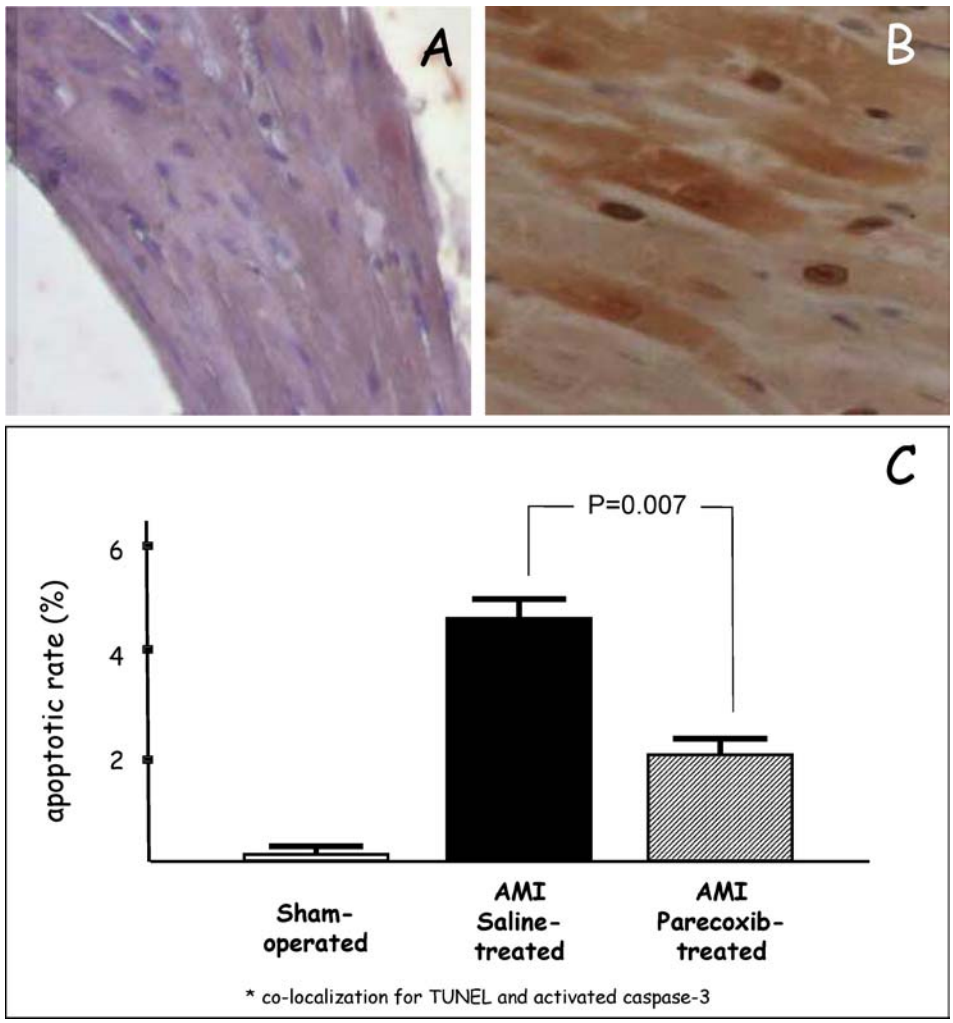
This study shows for the first time that COX-2 inhibition by parecoxib significantly reduces ongoing cardiomyocyte loss due to apoptosis during the first week after AMI in a model of permanent infarct-related artery occlusion.

The use of COX-2 inhibitors in animal models of AMI due to coronary ligation had shown beneficial effects in terms of more favourable remodelling, however the underlying mechanisms were unclear. In this study we hypothesized and tested whether the beneficial effects of COX-2 inhibition were due to inhibition of peri-infarct apoptosis.

The role of COX-2 in ischemic heart disease and tissue response to ischemia is still highly debated.[4] The use of COX-2 inhibitors (along with all non-steroidal anti-inflammatory drugs) has become off-limit for patients with or at risk for acute coronary syndromes.[7] The reason why COX-inhibition may favor atherothrombosis is yet completely clear. An action on endothelium and platelets is suggested. Nevertheless the mechanism by which COX-2 inhibition may increase the risk of atherothrombosis may be completely dissociated from the potential beneficial effects of COX-2 inhibition in tissue response to ischemia and post-infarction remodelling. In this study acute myocardial ischemia was induced by coronary ligation and a COX-2 inhibitor was started only 24 h after occlusion in order to lessen the influence on acute thrombotic/fibrinolytic balances and acute inflammatory reaction to necrosis. It is not surprising that COX-2 inhibition started 24 h after coronary ligation did not affect infarct size, whereas it is relevant that the amounts of cells undergoing death by apoptosis at day 7 were less. A major limitation of this study is the lack of assessment of functional consequences of reduced apoptosis, nevertheless the aim of the study was to investigate the pathophysiologic mechanisms associated with a functional improvement already shown by other studies,[2,3] and studies that have inhibited apoptosis chronically have shown improved functional outcome.[8] Another limitation is the unknown mechanism by which COX-2 inhibition prevents apoptosis, and further studies may be required.

In conclusion, while results of animal experimental studies should not be translated to clinical practice without appropriate validation, the findings of a beneficial effect of COX-2 inhibition on apoptosis may have strong

Figure 2. COX-2 and apoptosis. COX-2 expression in the peri-infarct area is shown at immunohistochemistry in panel A. Panel B shows diffuse cytoplasmic staining for activated caspase-3 and many TUNEL positive (dark brown) nuclei. Panel C shows a histogram of the main results of the study. Rats who had received parecoxib had significantly lower apoptotic rates in the peri-infarct myocardium than those treated with normal saline.



implications for the prevention of post-ischemic cardiac remodeling and heart failure.

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