Administration of the selective cyclooxygenase (COX)-2 inhibitor etodolac prolongs cardiac allograft survival in a mouse model

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Received March 8, 2010; Accepted July 21, 2010

DOI: 10.3892/mmr.2010.339

Abstract. Etodolac, a selective cyclooxygenase-2 (COX-2) inhibitor, is a non-steroidal anti-inflammatory drug. COX-2 is a key factor in the progression of inflammation. Although inflammation is an essential pathologic feature of cardiac allograft rejection, the role of COX-2 in this process remains unclear. The aim of this study was to investigate the expression of COX and the effects of etodolac in a mouse cardiac allograft model. Balb/c mice (H-2d) were used as recipients and C57BL/6 (H-2b) mice as heart donors. Heart function was evaluated daily after transplantation by regular abdominal palpation of the heart and by laparotomy in cases where the beating became weak. Rejection was defined as total cessation of cardiac muscle contraction. COX-2 expression was analyzed by immunohistochemistry. Cardiac isograft was well tolerated (>150 days, n=5), while non-treated cardiac allograft was rapidly rejected (mean 10.9±2.4, n=7). In the etodolac-treated cardiac allograft (10 mg/kg/day by hypodermic injection), survival was extended to 18.53±2.1 days (n=7). The necrotic area and the grade of COX-2 immunostaining were more significantly reduced in the etodolac-treated cardiac allograft than in the non-treated cardiac allograft at day 14. These results indicate that etodolac contributes to protection against rejection after heart transplantation. Etodolac could therefore be used to suppress graft rejection by means of its anti-inflammatory properties.

Introduction

Myocardial inflammation constitutes a major component of the pathologic changes observed during cardiac allograft rejection. Prostaglandins (PGs), along with leukotrienes and lipoxins, are lipid mediators which contribute to the vasodialation, edema and plasma protein leakage that occur during the inflammatory response (1).

The metabolism of arachidonic acid by the cyclooxygenase (COX) pathway generates eicosanoids, which have been implicated in the pathogenesis of a variety of human diseases, including heart disease. COX is the first enzyme in the pathway responsible for producing PGs and thromboxane from arachidonic acid. There are two isoforms of COX, COX-1 and COX-2. COX-1 occurs in tissues and cells and works to protect the cell. COX-2 is expressed momentarily and strongly on growth factors, promotors and certain endotoxins, and is involved in inflammation, cell growth and differentiation. COX-1 is constitutively expressed in most tissues, whereas COX-2 is induced in response to pro-inflammatory cytokines and stress (2).

It is believed that COX-2 is induced during both acute and chronic inflammatory responses, and is primarily responsible for the PG synthesis that ensues. This has led to the development of drugs that selectively inhibit PG production by COX-2, thus preventing adverse consequences, such as gastric ulcers, that may result from the inhibition of COX-1 (3). The PGs in particular play a role in the pathogenesis of inflammation involving cell-mediated immune responses, such as those that occur in rheumatoid arthritis and allograft rejection.

In a cardiac allograft model, it has been reported that COX-2 mRNA and enzyme proteins are up-regulated during cardiac allograft rejection (1). Therefore, the present study was designed to investigate whether COX-2 is expressed in a mouse cardiac allograft model and whether the selective COX-2 inhibitor etodolac helps inhibit myocardial inflammation during cardiac allograft rejection.

Materials and methods

Animals. Balb/c (H-2d) and C57BL/6 (H-2b) mice were obtained from the original animal facilities at Claude Bernard University, Edouard Herriot Hospital.

Heart transplantation. Surgical procedures were performed under anesthesia by intra-peritoneal injection of Hypnomidate

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Key words: cyclooxygenase-2, etodolac, mice cardiac allograft, survival

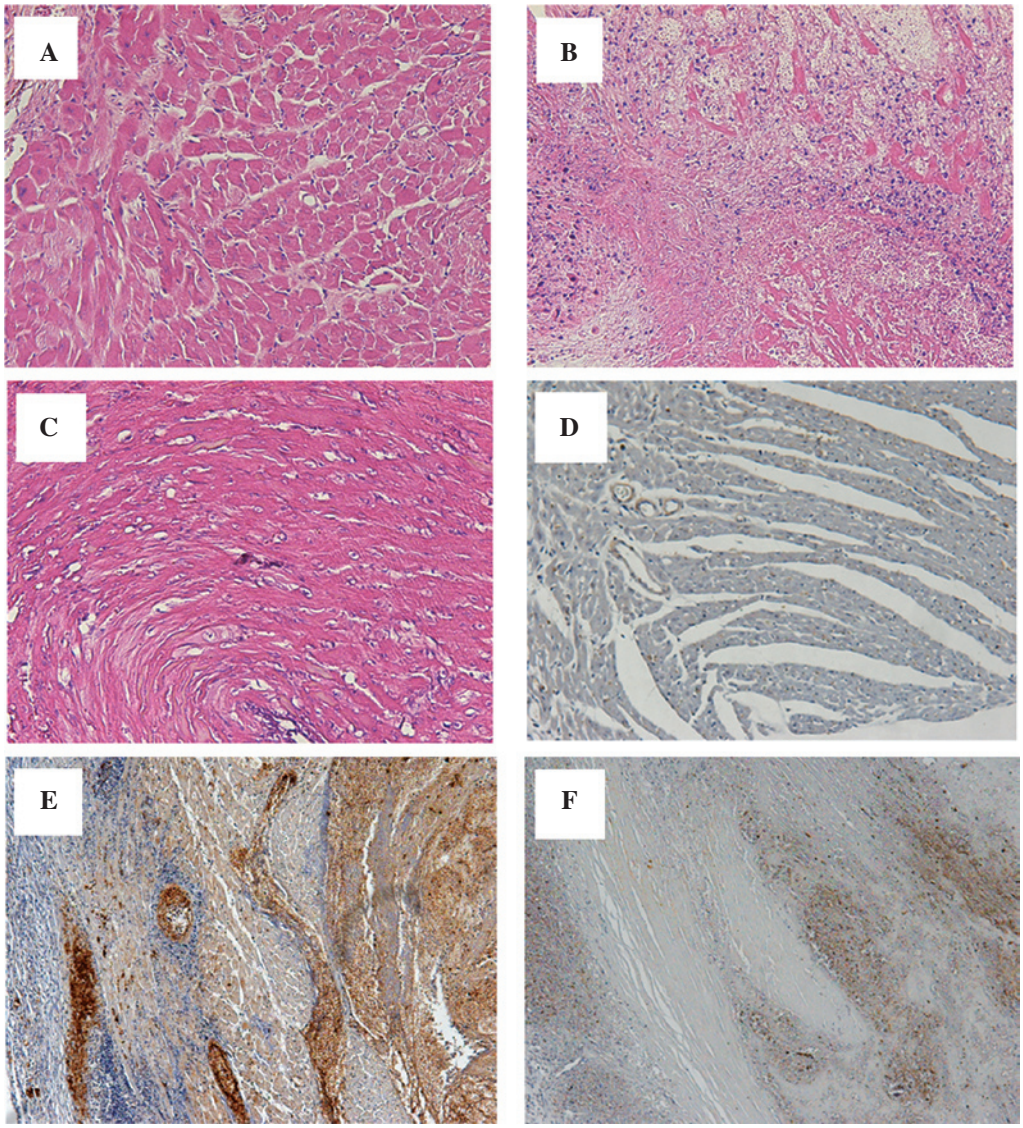

Figure 1. H&E staining (A-C) and COX-2 immunohistochemistry (D-F) from samples collected on day 14. (A) H&E staining of the cardiac isograft revealed normal tissue architecture, with no damaged myocardia. (B) In the non-treated cardiac allograft, extensive lymphocyte infiltration was observed, and almost all the myocardia were damaged and destroyed. Complete rejection occurred. (C) In the etodolac-treated cardiac allograft, some areas were infiltrated by lymphocytes and some myocardia were damaged and destroyed. However, the majority of myocardia were preserved. (D) In the cardiac isograft, slight COX-2 immunostaining was observed in endothelial cells. (E) In the non-treated cardiac allograft, marked COX-2 immunostaining was observed in macrophages, damaged cardiomyocytes and endothelial cells, and inflammatory infiltrate. (F) In the etodolac-treated cardiac allograft, COX-2 immunostaining was most apparent in the infiltrating macrophages, with decreased immunostaining of cardiac myocytes.

(20 mg/kg) and Midazoram (5 mg/kg). Vascularized heterotopic heart grafts from B6 (H-2b) mice were placed in the abdomen of BALB/c (H-2d) recipient mice and anastomosed to the abdominal aorta and vena cava, as we described previously (4). Heart function was evaluated daily after transplantation by regular abdominal palpation of the transplanted heart and by laparotomy in cases where the beating became weak. Rejection was defined as total cessation of cardiac muscle contraction.

Selective COX-2 inhibitor administration. The selective COX-2 inhibitor etodolac was obtained from Nippon Shinyaku Co., Ltd. (Kyoto, Japan). Recipient mice were injected subcutaneously with etodolac (10 mg/kg/day by hypodermic injection) daily from day 0 as the treatment group. Control group mice were treated with the same volume of phosphate-buffered saline (PBS). Etodolac has anti-inflammatory effects with a better safety profile for the stomach than non-selective non-steroidal anti-inflammatory drugs (NSAIDs) (5).

Histology and immunohistochemistry. Cardiac allograft tissue specimens were collected on day 14 and preserved in 10% formalin, embedded in paraffin, serially sectioned onto microscope slides at a thickness of 4 μm, and finally deparaffinized. For routine histologic examination, sections were stained with H&E to determine the extent and severity of rejection according to the International Society of Heart and Lung Transplantation classification (6). Immunohistochemical staining was performed with the VectaStain avidin-biotin peroxidase complex kit (Vector Laboratories, CA, USA) as previously described (7). Primary polyclonal antibodies against mouse COX-2 (1:50 dilution in PBS) (Cayman Chemical, MI, USA) and control PBS were used.

Results

Allograft survival. Cardiac isograft was well tolerated (>150 days, n=5), while non-treated cardiac allograft was rapidly rejected (mean 10.9±2.4, n=7). In the etodolac-treated cardiac allograft (10 mg/kg/day by hypodermic injection), survival was extended to 18.53±2.1 days (n=7).

H&E staining. H&E staining of the cardiac isograft tissues revealed normal tissue architecture, with no damaged myocardia (Fig. 1A). In the non-treated cardiac allograft, extensive lymphocyte infiltration was observed and almost all the myocardia were damaged and destroyed. Complete rejection occurred (Fig. 1B). In the etodolac-treated cardiac allograft, some areas were infiltrated by lymphocytes and some myocardia were damaged and destroyed. However, the majority of myocardia were preserved (Fig. 1C).

Immunohistochemistry. In the cardiac isograft tissues, slight COX-2 immunostaining was observed in endothelial cells (Fig. 1D). In the non-treated cardiac allograft, marked COX-2 immunostaining was observed in macrophages, damaged cardiomyocytes and endothelial cells, and inflammatory infiltrate (Fig. 1E). In the etodolac-treated cardiac allograft, COX-2 immunostaining was most apparent in the infiltrating macrophages, with decreased immunostaining of cardiac myocytes (Fig. 1F).

Discussion

Myocardium inflammation (leukocyte infiltration and interstitial edema) along with contractile dysfunction and death of cardiac muscle cells are hallmarks of acute cardiac allograft rejection (8,9). Along with cytokines, histamine and kinins, the eicosanoids are a family of lipid mediators that contribute to the vasodialation and leakage of plasma proteins and fluid into the interstitial space, which occurs during the acute inflammatory response in the myocardium and in other tissues (10).

COX, which produces prostanoids from arachidonic acid, is the principal target of NSAIDs. Recently, two COX isoforms, COX-1 and COX-2, were identified. Both the COX-1 and COX-2 enzymes are transformed from cell membrane phospholipids to arachidonic acid by phospholipaseA_2, and then transform arachidonic acid to PGH_2 through PGG_2. COX-1 is present in most tissues and is involved in the physiological production of PGs for the maintenance of normal homeostasis. COX-2 is well-established to participate in prostanoid production and is implicated in the processes of disease and expressed in inflammatory sites that often have harmful effects. COX-2 expression is therefore associated with pain, fever, lipopolysaccharide stimulation, ischemia/reperfusion injury and allograft rejection (11).

In cardiovascular diseases, COX-2 is known to have a cardioprotective protection effect that alleviates ischemia/reperfusion injury, mediating the late phase of pre-conditioning (12). Since acute cardiac rejection of transplanted heart is known to cause serious myocardial inflammation, COX-2 is enhanced in rejected cardiac allografts (1). The inhibition of COX-2 prolongs cardiac allograft survival and reduces myocardial damage and inflammation during acute cardiac allograft rejection in a rat model (10).

Several studies corroborate our data. Yang *et al* reported that COX-2 expression is enhanced in the myocardium during cardiac allograft rejection by immunohistochemistry in a rat model (1). Ningsheng *et al* also reported that the administration of the COX-2 inhibitor DFU (5 mg/kg/day by intraperitoneal injection) prolongs cardiac allograft survival (from 6.3±0.5 to 12.6±2.6 days, P=0.01) and reduces myocardial damage and inflammation during acute cardiac allograft rejection in a Lewis rat model (10). In a mouse model, Ogawa *et al* reported that the COX-2 inhibitor meloxicam (0.1 mg/kg/day intraperitoneal injection) suppressed inflammation and fibrosis in cardiac allografts. However, they found that non-treated cardiac allografts were acutely rejected (7.8±0.4 days, n=6) and that COX-2 inhibitor did not prolong cardiac allograft survival (7.5±4.2 days, n=6) (13).

It is well known that meloxicam and etodolac are selective COX-2 inhibitors. There are therefore several possible reasons for the differing results of the abovementioned studies. First, etodolac has higher selectivity than meloxicam (14,15). Second, etodolac is not associated with a statistically increased risk of acute myocardial infarction compared to other COX-2 inhibitors (16). Etodolac has been associated with cardionegative or cardioprotective effects in studies comparing them to NSAIDs (17). Finally, etodolac showed the highest UD(50) value and safety index as compared to NSAIDs and meloxicam in arthritic rats (5). These differences may be responsible for the prolongation of cardiac allograft survival by etodolac.

In conclusion, the administration of etodolac prolongs cardiac allograft survival and reduces myocardial damage and inflammation during acute cardiac allograft rejection.

The present study demonstrates that the selective COX-2 inhibitor etodolac, administered by subcutaneous injection, prolongs cardiac allograft survival and reduces myocardial damage and inflammation during cardiac allograft rejection in a mouse model. These results suggest that, with further study, etodolac may emerge as another agent in the therapeutic armamentarium for the treatment of patients undergoing heart transplantation.

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