Cyclooxygenase-2 Inhibitor Treatment Improves Left Ventricular Function and Mortality in a Murine Model of Doxorubicin-Induced Heart Failure

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Background—Progression of heart failure after initial myocardial injury is mediated in part by various redundant inflammatory mediators, including the widely expressed cyclooxygenase-2 (COX-2). Because COX-2 inhibitors are useful in treating many inflammation-mediated diseases, we asked whether COX-2 inhibition can attenuate heart failure progression.

Methods and Results—Heart failure was experimentally induced in 100 mice by administration of doxorubicin (4 mg · kg\(^{-1}\) · wk\(^{-1}\) for 6 weeks). Beginning at day 42, mice were fed daily with either COX-2 inhibitor–containing mice chow (n=50) or plain mice chow (controls; n=50). Left ventricular ejection fraction was evaluated as a measure of heart failure by a novel method of transthoracic echocardiography (with intravascular ultrasound catheters) at baseline and on days 42, 56, and 70. From baseline to study termination, left ventricular ejection fraction in COX-2 inhibitor–treated mice decreased significantly less than in control mice (9% versus 29%, \(P<0.01\)). Mortality was significantly lower for COX-2 inhibitor–treated mice than for control mice (18% versus 38%, \(P<0.01\)). These results were confirmed in a revalidation study in COX-2 inhibitor–treated mice (n=25) and controls (n=25). That study revealed that the hearts from control mice weighed roughly the same as hearts from COX-2 inhibitor–treated mice but showed more extensive signs of cardiomyopathy (as determined by pathological analysis by an independent, blinded observer) and higher levels of COX-2 proteins (as determined by immunoblotting [6442±1635 versus 4300±2408 arbitrary units, \(P<0.022\)].

Conclusions—COX-2 inhibitors can attenuate the progression of heart failure in a murine model of doxorubicin-induced heart failure. (Circulation. 2004;109:1428-1433.)

Key Words: heart failure ■ inhibitors ■ imaging

Congestive heart failure (CHF) is one of the most serious cardiovascular diseases in adults, especially the elderly, and one of the most costly. More than 4 million Americans have CHF, and the incidence is on the rise.\(^1\) The disease is increasing with aging of the population and is currently the most common cause of hospital admission among the elderly. More than $5 billion per year is spent on the treatment of CHF.\(^2\)

CHF is a systemic disease characterized by impaired cardiac contractility leading to decreased cardiac output, increased neuroendocrine and inflammatory cytokine activity, and ultimately increased cardiac filling pressures and congestion or edema. A wide spectrum of injurious processes, ranging from ischemia to myocardial toxin–induced damage to volume/pressure overload to genetic abnormalities, creates a milieu in which multiple neuroendocrine, humoral, and inflammatory feedback loops are deranged and cardiac remodeling occurs. Compensatory mechanisms exacerbate the detrimental remodeling process by shifting the cardiac workload from dying myocytes to healthier, contracting cells, which in turn begin to weaken and die. From this vicious cycle arise the classic signs and symptoms of CHF.

Although their roles are poorly understood, necrosis and apoptosis appear to contribute greatly to the progression of heart failure. A number of necrotic and apoptotic promoting factors, including proinflammatory cytokines and immune mediators, as well as early growth response genes, have already been implicated in heart failure, and a large body
of evidence now suggests that their ability to further weaken the failing heart is mediated by inflammatory mechanisms.\textsuperscript{7–9}

Whereas most current therapies for CHF target the mechanical, hemodynamic, arrhythmic, or neurohormonal aspects of the disease, few, if any, target inflammation at the myocardial level. This picture is changing, however, as potential targets (eg, cytokines, matrix metalloproteinases, and cyclooxygenase [COX]-2) are identified. Selected cytokines and other inflammatory mediators have been shown to increase in CHF, especially at the tissue level.\textsuperscript{1,6–10} Matrix metalloproteinases, which are involved in many types of inflammatory and reparative responses, appear to be involved in adverse remodeling of the myocardium in CHF.\textsuperscript{11–13} Induction of the powerful inflammatory mediator COX-2 in the myocardium has been associated with heart failure,\textsuperscript{10} which suggests that COX-2 may be a key element in the final pathway of the inflammatory process. However, it remains unclear whether COX-2 inhibition is better achieved by selective or nonselective means. In an animal model of cardiopulmonary dysfunction, pigs showed improvement when treated with indomethacin, a nonselective COX-1 and COX-2 inhibitor.\textsuperscript{14} Conversely, in a more recent study in humans, indomethacin caused adverse hemodynamic and renal effects.\textsuperscript{15} To clarify this question, we used a mouse model of drug-induced heart failure to test the hypothesis that the progression of heart failure can be attenuated by selective COX-2 inhibition after initial injury and that this may have a beneficial effect on cardiac function and mortality.

**Methods**

**Animals and Materials**

CD-1 mice were obtained from Charles River Laboratories (Wilmington, Mass). The COX-2 inhibitor used in this study (Merck Frosst Tricyclic) was a gift from Merck Frosst Inc (Pointe-Claire-Dorval, Quebec, Canada). Control mice chow and the same chow premixed with the COX-2 inhibitor were obtained from Merck Frosst Inc. Doxorubicin was purchased from a local pharmacy.

**Heart Failure Induction Protocol**

Heart failure (ie, left ventricular [LV] dysfunction) was induced experimentally in 100 mice by intraperitoneal injections of the cardiotoxic agent doxorubicin (4 mg/kg) weekly for 6 weeks (Table 1). This previously described, reproducible model causes progression to end-stage heart failure in most cases.\textsuperscript{16}

**COX-2 Inhibitor Treatment Protocol**

On the last day of the heart failure induction protocol (day 42), the mice were randomly divided into 2 groups, 1 to receive the COX-2 inhibitor (n=50) and the other to serve as a control group (n=50). The treatment protocol is described in Table 1. The COX-2 inhibitor we used, Merck Frosst Tricyclic, is a selective inhibitor of COX-2 that has no significant activity against COX-1 when administered in clinically effective concentrations.\textsuperscript{17} The COX-2 inhibitor was mixed with mice chow at a concentration previously determined in murine pharmacokinetic studies conducted by Merck to be equivalent to a relatively high therapeutic dose in humans (≈0.1 mg/g body weight per day).\textsuperscript{17} The half-life of Merck Frosst Tricyclic in mice is ≈12 hours, and 87\% of the drug is protein bound.\textsuperscript{17}

**Functional Evaluation**

To evaluate heart failure progression and the effect of treatment over time, LV ejection fractions (LVEFs) and LV dimensions were measured by noninvasive transthoracic echocardiography with intravascular ultrasound (IVUS) catheters at baseline and at days 42, 56, and 70 (Table 2). IVUS catheters were used because the frequencies emitted by more conventional ultrasound probes (<15 MHz) severely limit visual resolution of the small structures of the rapidly beating mouse heart. The 6F IVUS catheter we used (Boston Scientific Scimed) has an axial resolution of 0.2 mm, a depth of penetration of >15 mm, and a wave-emitting frequency of 20 MHz, features that allow excellent visualization of murine cardiac structures. Figure 1 provides a representative set of IVUS images acquired from a control mouse.

The IVUS protocol was as follows. First, each mouse was given ketamine and xylazine to induce light anesthesia. Next, the chest was shaved, and mineral oil was applied to create an acoustic interface. Each mouse was then placed on a warming blanket to prevent hypothermia and bradycardia. The IVUS catheter was placed, with the transducer, over the left sternal border and rotated to obtain short- and long-axis images of the LV. Images were recorded on Super-VHS videotape and later examined by a single observer using an offline program for analyzing and interpreting human clinical echocardiograms (Digisonics Echo Interpretation System; Digisonics Inc). The observer was blinded to the treatment group. Accuracy of distance measurement was ensured by calibrating distance measured to the known measurement markings on the IVUS image. The reproducibility of LVEF measurements by IVUS was evaluated by having a single observer measure the LVEF in 100 normal mice. There were no fatal complications due to anesthesia or echocardiography.

**Revalidation Study**

To validate our initial findings, we performed a second study in which the effects of COX-2 inhibitor treatment on the heart failure model were assessed histopathologically and biochemically. In brief, half the original number of mice were treated just as in the initial study: 25 doxorubicin-treated mice received COX-2 inhibitor, and 25 doxorubicin-treated mice (controls) did not. In addition, 5 mice were given neither doxorubicin nor COX-2 inhibitor, and 5 were given only the COX-2 inhibitor. On day 70, the mice were killed and necropsied.
Necropsy, Histopathology, and Tissue Preparation

At necropsy, hearts were explanted, washed of blood, weighed within 7 seconds, rapidly frozen in liquid nitrogen, and sent for histopathological examination and for biochemical assay as described below. Frozen whole-heart tissue samples were homogenized in PBS containing a protease inhibitor cocktail (Complete; Roche) and then centrifuged at 3500 rpm. The resulting supernatants were assayed for protein concentration by the Bradford method with a Bio-Rad Protein Assay kit and then subjected to further analyses.

Western Blot Analysis

Expression of COX-2 protein was assessed by Western immunoblot analysis as described previously.18 Tissue lysate (75 µg) was loaded into each lane of a mini gel (Bio-Rad) and then subjected to polyacrylamide gel electrophoresis across a 4% to 15% SDS gradient. COX-2 signals were identified with a monoclonal antibody specific for COX-2 (Cayman Chemical), visualized with an enhanced chemiluminescence system (ECL; Amersham Pharmacia Biotech), and quantified by image scanning densitometry.

Statistical Analysis

The effect of COX-2 inhibitor treatment on LVEF was analyzed by ANOVA. The statistical model used was 2-way ANOVA in which the main statistical parameters were treatment and time. The outcomes of the 2 treatments at each of the 4 time points studied were compared. The continuous end points of interest (mortality, LVEF, and mass) were analyzed with the General Linear Models procedure included with the commercially available SAS/STAT software package (SAS Institute Inc). The relation between mass as determined by IVUS and mass as determined at necropsy was assessed with the Pearson product-moment correlation coefficient. Probability values less than 0.05 were considered statistically significant. Post hoc comparisons were adjusted by Bonferroni correction.19 Differences in mortality were assessed by chi-square analysis. Data are presented as mean±SD for continuous variables and as percentages for categorical variables.

Results

Functional Evaluation

As summarized in Table 3, mean LVEF in COX-2 inhibitor–treated and control mice decreased similarly from baseline to the end of the heart failure induction period (day 42). From baseline to day 56, however, the decrease in mean LVEF between the 2 groups was significantly different: 13% in the COX-2 inhibitor–treated mice versus 28% in controls (P<0.01). From baseline to day 70, the difference was even greater: a 9% decrease in COX-2 inhibitor–treated mice versus 29% in controls (P<0.01). Diastolic dimension did not change in COX-2 inhibitor–treated mice but did increase in control mice (Table 3).

Because IVUS of the mouse heart is a new technique, we tested the reproducibility of our echocardiographically determined LVEF measurements. Reproducibility was confirmed by a single observer, who obtained a mean LVEF of 71±4.8% in 100 normal mice. We also tested the accuracy of the technique in determining myocardial mass. First, we compared the myocardial mass determined by echocardiography with the mass as determined by IVUS.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean LVEF, %</th>
<th>Diastolic Dimension, mm (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2 inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1 (baseline; n=50)</td>
<td>70±5</td>
<td>4.15 (3.6–4.6)</td>
</tr>
<tr>
<td>Day 42 (n=50)</td>
<td>63±5</td>
<td>4.18 (3.7–4.6)</td>
</tr>
<tr>
<td>Day 56 (n=45)</td>
<td>61±6*</td>
<td>4.24 (3.8–4.6)</td>
</tr>
<tr>
<td>Day 70 (n=35)</td>
<td>64±5†</td>
<td>4.24 (3.6–4.6)</td>
</tr>
<tr>
<td>No treatment (controls)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1 (baseline; n=50)</td>
<td>72±4</td>
<td>3.78 (3.4–4.5)</td>
</tr>
<tr>
<td>Day 42 (n=46)</td>
<td>60±8</td>
<td>3.77 (3.4–4.3)</td>
</tr>
<tr>
<td>Day 56 (n=29)</td>
<td>52±10*</td>
<td>4.01 (3.4–4.6)</td>
</tr>
<tr>
<td>Day 70 (n=27)</td>
<td>51±7†</td>
<td>4.12 (3.6–4.6)</td>
</tr>
</tbody>
</table>

n indicates No. of mice alive at each time point.
*P<0.01 vs control value.
†P<0.01 for comparison of difference between value at baseline and at end of treatment.
raphy with the myocardial mass determined by weight at necropsy (n=36). This yielded a correlation coefficient of 0.66 (P=0.0001). Second, we compared the myocardial mass determined by echocardiography with that determined by the septum-plus-free-wall-thickness method at necropsy (n=34). This yielded a correlation coefficient of 0.73 (P=0.0001). Together, these findings indicated that our echocardiographic LVEF findings were reproducible and that the echocardiographically derived mass measurements were accurate.

**Mortality and Survival**

During the COX-2 inhibitor treatment period (days 42 to 70), mortality was 18% (9/50) for COX-2 inhibitor–treated mice versus 38% (19/50) for the control mice (P<0.01). Most deaths were preceded by the development of ascites and edema. There was a clear association between lower LVEF (<45%) and increased mortality. The death rate, as evaluated by Kaplan-Meier analysis, was significantly higher in the control group than in the COX-2 inhibitor–treated group (P<0.001; Figure 2).

**Revalidation Study Results**

The results of our revalidation study confirmed our initial findings. Mortality for COX-2 inhibitor–treated mice was significantly lower than for control mice (28% [7/25] versus 56% [14/25]; P<0.002; Figure 3). Histopathologically, the hearts of control mice showed signs of cardiomyopathy, as determined by an independent, blinded observer. At necropsy (n=11), 10 control mice had ascites, 7 had a lobulated enlarged liver indicating liver congestion, and 5 had foamy lungs indicating pulmonary edema. In comparison, hearts from COX-2 inhibitor–treated mice (n=18) showed few signs of cardiomyopathy, and there was 1 instance of ascites. These findings were not evaluated quantitatively. Mean heart weights were similar (0.18±0.5 g in treated mice versus 0.18±0.4 g in controls) and not statistically different. However, mean COX-2 protein expression was higher in untreated controls than in the COX-2 inhibitor–treated mice (6442±1635 versus 4300±2408 arbitrary units; P<0.022; Figure 4). In the 5 mice that received neither doxorubicin nor COX-2 inhibitor, there were no deaths or abnormal pathological findings, and in the 5 that received only the COX-2 inhibitor, there was 1 death and no abnormal pathological findings.

**Discussion**

In the studies reported here, we have shown that the progression of heart failure in a mouse model was attenuated by selective COX-2 inhibition after initial drug-induced myocardial injury and that this had a beneficial effect on LVEF and mortality. Our studies were prompted by the growing general interest in using inflammatory mediators as a treatment target for heart failure. Immunomodulating agents, such as a serine elastase inhibitor and vesnarinone, have produced both functional and histological improvement in animal models of heart failure.7,20 Matrix metalloproteinases have been implicated in many inflammatory syndromes and in adverse cardiac remodeling and have been shown to be upregulated in the failing heart.11,21–23 It is now known that immune cells and inflammatory mediators localize in the failing myocardium, where they modulate myocyte function and very likely affect remodeling of the myocardial architecture.24 It is also known that end-stage heart failure is associated with a systemic inflammatory syndrome.25 Chronic, low-grade inflammation is often present in the chronically failing human myocardium, independent of the cause of heart failure, and contributes significantly to the structural deterioration that leads to reduced global function.7

There is sparse but intriguing evidence in the literature for the involvement of COX-2 in heart failure progression. In humans, COX-2 has been found to be expressed in myocardium damaged by ischemia or dilated cardiomyopathy but not in normal cardiomyocytes.19 In pigs, hemodynamic responses similar to those seen in cases of heart failure have been induced by the infusion of the inflammatory mediators tumor
necrosis factor-α and interleukin-1α and attenuated by the administration of indomethacin, a nonselective COX-1 and COX-2 inhibitor. In a histopathological study, myocardial tissue samples from patients with end-stage heart failure attributed to various causes contained abundant COX-2 protein and exhibited a high specificity for anti-COX-2 antibody staining. In the same study, myocytes and inflammatory cells in ischemic and fibrotically scarred, as opposed to morphologically normal, areas of myocardium expressed COX-2 mRNA and protein in abundance.

We examined the effects of a COX-2 inhibitor on experimentally induced heart failure in mice. The improvement in LV function and survival that we saw in our model suggests that COX-2 activity may not be redundant like that of other proinflammatory cytokines but may instead be a basic, common factor in the progression of heart failure, the inhibition of which cannot be easily circumvented. Alternatively, because there was still some mortality in the COX-2 inhibitor–treated group of mice, the data may also suggest that COX-2 is important but, like many other deleterious factors in heart failure, not a basic, common factor. The present results thus suggest that COX-2 inhibition may show promise in improving some forms of heart failure. COX-2 inhibitors are already known to modulate the immune response in various disease states and are they are currently being used clinically in the treatment of arthritis and autoimmune disease. However, selective COX-2 inhibitors have been implicated in causing significant salt and water retention, leading to edema in some patients with heart failure, so this must be considered too, especially when coexistent renal dysfunction is present.

The present data support the hypothesis that selective COX-2 inhibition attenuates the progression of doxorubicin–induced heart failure by downregulating cardiac COX-2 expression and consequently the inflammatory response of myocardial cells to injury. More than 1 group has reported that COX-2 inhibition therapy can aggravate doxorubicin–induced cardiomyopathy when given concurrently with doxorubicin. In the present case, however, COX-2 inhibition treatment was initiated after heart failure had been established by doxorubicin. Therefore, the effects of the COX-2 inhibitor cannot be related to an interaction with the effects of the doxorubicin. Thus, in the case of doxorubicin myocardial toxicity, it appears that COX-2 may have an initial adaptive role in attenuating the injury caused by the anthracycline, but after the insult is removed, the progression of the heart failure may be worsened by COX-2 induction. It is this progressive phase after the initial insult that may be positively impacted by COX-2 inhibition. It is possible, given that COX-2 overexpression has been found in various forms of heart failure, that this effect may occur regardless of the cause of the initial insult. This phenomenon may be analogous to other neurohormonal and inflammatory processes in which the effects are initially adaptive to attenuate injury during the stress but later become maladaptive, leading to disease progression.

Thus, we believe that COX-2 inhibition exerts a positive effect on the progression of heart failure after the initial insult has occurred in the experimental murine model we have used. The delay of treatment until after the toxic insult in the present study was designed to evaluate whether COX-2 inhibition may be useful in attenuating relentless progression of heart failure after an injury, such as that associated with doxorubicin administration. Further studies are needed to determine whether these results can be translated to the more complex human subject with heart failure and coexistent conditions, such as chronic renal insufficiency.

Conclusions
Anti-inflammatory therapy with a COX-2 inhibitor holds promise for hindering the progression of heart failure after an initial myocardial insult. More studies are now needed to confirm the present findings, to determine the optimal dose of COX-2 inhibitor that is most therapeutically effective with minimal side effects, and to determine whether COX-2 inhibition provides similar protection against heart failure due to other causes.

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