Role of acetaminophen in acute myocardial infarction

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Acetaminophen, the active ingredient in Tylenol, is a widely used drug that is well known for its analgesic and antipyretic properties. However, the cardiovascular effects of acetaminophen have not been fully assessed. Data are particularly lacking regarding the effect of acetaminophen in the setting of an AMI. Recently, the use of acetaminophen has been studied as a potential adjunct to reperfusion therapy for AMI (6, 16, 27). The results regarding the efficacy of acetaminophen in this setting are inconclusive and, in some cases, contradictory.

Using both large (ovine) and small (rabbit) collateral-deficient animal models of reperfused AMI, we studied the effects of acetaminophen on myocardial salvage, myocardial blood flow, ventricular function, hemodynamics, and apoptotic cell death. In the setting of an AMI, we sought to identify any potential harmful or cardioprotective effects of the drug.

MATERIALS AND METHODS

Surgical protocol. Sixteen Dorset male hybrid sheep weighing 30–45 kg and twenty-two New Zealand White male rabbits weighing 3–4 kg were used in this study. Animals were treated under experimental protocols approved by the University of Pennsylvania’s Institutional Animal Care and Use Committee and in compliance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publications No. 85-23, Revised 1996).

Anesthesia was induced with thiopental sodium (10–15 mg/kg iv), and sheep were intubated, anesthetized with isoflurane (1.5–2%), and ventilated with oxygen (Drager anesthesia monitor, North American Drager, Telford, PA). Fluid-filled catheters were placed in a femoral artery and internal jugular vein for the continuous measurement of blood pressure and the administration of intravenous medications. A Swan-Ganz catheter (131h-7F, Baxter Healthcare, Irvine, CA) was introduced into the pulmonary artery through the internal jugular vein, and a high-fidelity pressure transducer (Spc-350S, Millar Instruments, Houston, TX) was inserted from the femoral artery into the left ventricle (LV). Animals underwent a left thoracotomy, and silicone vascular loops (Quest Medical Allen) were placed around the left anterior descending artery and its second diagonal branch 40% of the distance from the apex to the base of the heart. Occlusion of these arteries at these locations has produced a well-characterized model of anterograde myocardial infarction in our laboratory (7, 8, 28).

In rabbits, anesthesia was induced with ketamine (50 mg/kg im), glycopyrrolate (0.2 mg/kg), and buprenorphine (0.05 mg/kg). Animals were intubated, anesthetized with isoflurane (0.25–1%), and ventilated with oxygen (Hallowell EMC model AWS, Pittsfield, MA). Fluid-filled catheters were introduced into a small auricular artery and vein and into the right jugular vein for the continuous measurement of blood pressure and the administration of intravenous medications. Additionally, a high-fidelity pressure transducer (SPR-524, Millar Instruments) was introduced through the right carotid artery into the
LV. Animals underwent a left thoracotomy, and a coronary snare was constructed by placing a pledgeted suture (4-0 silk, U.S. Surgical, Norwalk, CT) around a large branch of the circumflex coronary artery at \(-50\%\) of the distance from base to apex of the heart.

A hyper-/hypothermia unit (Medi-Therm III, Gaymar Industries, Orchard Park, NY) was used to maintain core temperature of 38–40°C in sheep and 39–41°C in rabbits. Arterial blood gases were measured in all animals, and the mean pH was \(7.40 \pm 0.07\) at the initiation of ischemia in rabbits and \(7.38 \pm 0.05\) in sheep.

**Experimental protocol.** Sheep and rabbits were divided into control or treatment groups. After instrumentation, baseline hemodynamic data and echocardiographic measurements were recorded. Sheep \((n = 8)\) and rabbits \((n = 11)\) in the control group received an intravenous infusion of a 10% ethanol solution as a vehicle. Sheep \((n = 8)\) in the treatment group received an intravenous infusion of 75 mg/kg of acetaminophen, dissolved in a 10% ethanol solution over 30 min. Rabbits \((n = 11)\) in the treatment group received an intravenous infusion of 15 mg/kg of acetaminophen, dissolved in a 10% ethanol solution. All animals received either vehicle or acetaminophen infusions 30 min before coronary artery occlusion. After repeat hemodynamic and echocardiographic measurements, heparin (10,000 U in sheep and 500 U/kg in rabbits) was administered. Additionally, a prophylactic intravenous anti-arrhythmic regimen of magnesium sulfate \((1 \text{ g iv})\), amiodarone \((150 \text{ mg iv})\), and lidocaine \((3 \text{ mg/kg iv bolus, and then } 2 \text{ mg/min infusion})\) was administered in the sheep model due to the high frequency of fatal arrhythmias after the induction of ischemia. The coronary snare were then tightened to produce an ischemic region of the LV. Ischemia was confirmed by a visible color change in the ischemic myocardial region, ST elevations on the electrocardiogram, and regional wall motion abnormalities on the echocardiogram. The ischemic period was 60 min in sheep and 30 min in rabbits. At the end of the ischemic period, coronary snares were loosened and the previously ischemic myocardium was reperfused for 3 h in all animals. The reperfused myocardium typically exhibited a visible hyperemic response. Hemodynamic measurements were recorded at 30-min intervals throughout the reperfusion period, and echocardiography was performed at 30 min and 165 min of reperfusion.

**Analysis of area at risk and infarct size.** At the completion of the protocol, the coronary snares were re-tightened, and vascular clamps were used to occlude the aorta, pulmonary artery, and inferior vena cava, and the right atrium was incised. Evans blue dye \((1 \text{ ml/kg; Sigma; St. Louis, MO})\) was injected via the left atrium to delineate the ischemic myocardial area at risk (AR). All animals were euthanized via an injection of KCl into the left atrium, and the heart was explanted. The LV was sectioned perpendicular to its long axis into 6–8 slices (Fig. 1A). The thickness of each slice was measured with a digital micrometer, and all slices were photographed. Infarct area was delineated by photographing and measuring the slices after 20 min of incubation in 2% triphenyltetrazolium chloride at 37°C (Fig. 1B). All photographs were imported into an image analysis program (Image-Pro Plus, MediaCybernetics; Silver Spring, MD), and computerized planimetry was performed. The AR is expressed as a percentage of the LV, and the infarct size \((I)\) is expressed as a percentage of the AR \((I/AR)\).

**Temperature and hemodynamic measurements.** Arterial blood pressure, LV pressure, heart rate, surface ECG, and rectal temperature were continuously monitored (Hewlett-Packard 78534C; Palo Alto, CA) throughout the protocol in both species. Hemodynamic, heart rate, and temperature measurements were recorded at baseline, postdrug (vehicle or acetaminophen) infusion, 20 min of ischemia, and every 30 min during reperfusion (Sonometrics, London, Ontario, Canada). Cardiac output was measured in triplicate at each time point in sheep. The maximal rate of LV pressure increase \((+dP/dt_{max})\) and decrease \((-dP/dt_{max})\) over time were calculated at each time point in all animals.

**Regional blood flow measurements.** In the sheep, fifteen-million color-coded, 15.5-μm diameter NuFlow Fluorescent microspheres (IMT, Irvine, CA) were injected to validate the absence of collateral circulation and completeness of ischemia during coronary occlusion and to evaluate any effect of acetaminophen on the microvasculature. Injections were made at baseline, 30 min after coronary occlusion, and at 10 and 165 min after the onset of reperfusion. Reference blood samples were taken at all time points. Myocardial samples and reference blood samples were analyzed with the use of flow cytometry for microsphere content by IMT. Regional perfusion was calculated by using the following formula: \(Q_m = (C_m \times Q_i)/C_s\), where \(Q_m\) is myocardial blood flow per gram (in ml·min\(^{-1}\)·g\(^{-1}\) of sample, \(C_m\) is microsphere count per gram of tissue in sample, \(Q_i\) is withdrawal rate of the reference blood sample (in ml/min), and \(C_s\) is microsphere count in the reference blood sample.

**Echocardiography.** Quantitative, two-dimensional, open-chest echocardiograms were performed at baseline, postdrug infusion, 20 min of ischemia, and 165 min of reperfusion in all animals. Images were obtained on a Philips 7500 ultrasound system using a 5-MHz (sheep) or 12-MHz (rabbits) transducer (model 77020A, Hewlett-Packard) with a custom-made offset device and recorded on 0.5-in. VHS videotape at 30 Hz (Panasonic AG-6300 VHS recorder). The transducer was placed at the cardiac apex, and two orthogonal long-axis views were recorded. LV end-systolic volume (LVESV) and LV end-diastolic volume (LVEDV) were calculated by using Simpson’s rule. Ejection fraction (EF) was calculated from LVESV and LVEDV.

**Sonomicrometry array localization.** Sonomicrometry array localization (SAL) is an imaging technique that uses small piezoelectric transducers to permanently label specific locations of myocardium \((14, 30)\). In 12 sheep, 14 transducers \((2 \text{ mm in diameter})\) were inserted into the myocardium of the LV free wall to form a grid on the anterior LV. This array consisted of five transducers within the planned infarction, three transducers at the edge of the infarct, and six transducers placed \(2\) to \(5\) cm cephalad to the infarct demarcation line.
similar in both sheep (23 ± 2% vs. 24 ± 2% with acetaminophen, \( P = 0.73 \)) and rabbits (22 ± 1% vs. 23 ± 2% with acetaminophen, \( P = 0.51 \)). In sheep, infarct size, expressed as a percentage of the AR (I/AR), was similar between the two groups (59 ± 7% vs. 64 ± 7% with acetaminophen, \( P = 0.64 \)). In rabbits, acetaminophen showed a trend toward limiting infarct size (34 ± 7% vs. 50 ± 8%, \( P = 0.16 \); Fig. 3).

Temperature, blood pressure, and heart rate. Core temperatures were not significantly different between groups at any time point in either species. Acetaminophen had no significant effect on heart rate at any time point in either species. The mean arterial blood pressure increased significantly (74 ± 2 to 88 ± 6 mmHg, \( P = 0.05 \)) after acetaminophen was given in sheep. There was no other blood pressure effect in sheep or rabbits (Table 1).

Ventricular function measurements. Ventricular function data are summarized in Table 2. In sheep, the infusion of acetaminophen before ischemia significantly increased cardiac output compared with controls (5.1 ± 0.3 vs. 3.5 ± 0.4 l/min, \( P < 0.01 \)). However, there was no other difference in cardiac output between groups at any other time point. In both sheep
and rabbits, LV systolic and diastolic function, as assessed by $+\frac{dP}{dt}_{\text{max}}$ and $-\frac{dP}{dt}_{\text{max}}$, were unaffected by the addition of acetaminophen (Fig. 4).

Apoptosis. The effect of acetaminophen on apoptotic cell death was studied in the rabbit experiments by the use of the ISOL technique, a highly specific test for the DNA cleavage fragments characteristic of apoptotic cell death (19). Despite the trend toward a reduction in infarct size in the acetaminophen group, there was no significant difference in the percentage of ISOL-positive cells between the two groups (6.0 ± 1.6% vs. 7.1 ± 2.2% with acetaminophen, $P = 0.73$).

Echocardiographic data. Baseline echocardiograms demonstrated normal wall motion of the anterolateral region of the heart in all animals. Images obtained after coronary occlusion confirmed a loss of apical contractility in the AR in all animals of both species. Elevations in LVESV and LVEDV were observed in all animals during the period of coronary occlusion, confirming significant ventricular dysfunction during the

Table 2. Parameters of ventricular function in sheep and rabbits undergoing ischemia and reperfusion

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<thead>
<tr>
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<th>Sheep</th>
<th>Rabbit</th>
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<tr>
<td></td>
<td>V</td>
<td>A</td>
</tr>
<tr>
<td>$+\frac{dP}{dt}_{\text{max}}, \text{mmHg/s}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>859±71</td>
<td>821±67</td>
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<tr>
<td>Post-drug</td>
<td>864±76</td>
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<tr>
<td>Ischemia</td>
<td>984±121</td>
<td>857±123</td>
</tr>
<tr>
<td>Rep-180</td>
<td>986±54</td>
<td>910±83</td>
</tr>
<tr>
<td>$-\frac{dP}{dt}_{\text{max}}, \text{mmHg/s}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>1,150±54</td>
<td>1,054±38</td>
</tr>
<tr>
<td>Post-drug</td>
<td>1,132±51</td>
<td>1,170±122</td>
</tr>
<tr>
<td>Ischemia</td>
<td>1,158±113</td>
<td>972±108</td>
</tr>
<tr>
<td>Rep-180</td>
<td>978±54</td>
<td>854±151</td>
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<tr>
<td>EF, %</td>
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</tr>
<tr>
<td>Base</td>
<td>44±2</td>
<td>41±2</td>
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<tr>
<td>Post-drug</td>
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<td>44±2</td>
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<tr>
<td>Ischemia</td>
<td>40±2</td>
<td>39±2</td>
</tr>
<tr>
<td>Rep-180</td>
<td>45±2</td>
<td>43±2</td>
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<tr>
<td>CO, l/min</td>
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<tr>
<td>Base</td>
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<tr>
<td>Post-drug</td>
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<td>5.1±0.3*</td>
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<tr>
<td>Ischemia</td>
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<td>3.9±0.4</td>
</tr>
<tr>
<td>Rep-180</td>
<td>3.5±0.3</td>
<td>3.3±0.2</td>
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Values are means ± SE. $+\frac{dP}{dt}_{\text{max}}$, maximal rate of left ventricular pressure increase over time; $-\frac{dP}{dt}_{\text{max}}$, maximal rate of left ventricular pressure decrease over time; Rep-180, 180 min after reperfusion; EF, ejection fraction; CO, cardiac output. *$P < 0.05$, between groups; †$P < 0.05$, from baseline values in acetaminophen-treated rabbits.

Fig. 4. Effects of acetaminophen and ethanol vehicle on LV systolic and diastolic function in sheep (A) and rabbits (B). Indexes of LV systolic maximal rate of LV pressure increase ($+\frac{dP}{dt}_{\text{max}}$) and diastolic pressure decrease over time ($-\frac{dP}{dt}_{\text{max}}$) function are shown at baseline, after infusion of ethanol or acetaminophen (Post-drug), at 20 min of ischemia, and after 165 min of reperfusion (Rep-165). LVESV and LVEDV, LV end-diastolic and end-systolic volumes, respectively. Comparisons between groups in either animal were not significantly different at any time point. *$P < 0.05$, from baseline values in acetaminophen-treated rabbits.
ischemic period (Fig. 5). Comparisons of LVESV, LVEDV, and EF between groups did not reveal any differences in animals receiving acetaminophen at any time point in either species.

Regional blood flow. Myocardial ischemia and the absence of collateral circulation were confirmed by the reduction in regional blood flow in the AR to $\leq 4\%$ of baseline values in both vehicle and acetaminophen-treated sheep. A hyperemic response was observed at the onset of reperfusion, but after 165 min of reperfusion, regional blood flow to the AR had fallen to $49 \pm 11\%$ in the vehicle group and $42 \pm 16\%$ in the acetaminophen group ($P = 0.74$). The addition of acetaminophen had no significant effect on regional blood flow at any time point (Fig. 6).

SAL. Normalized FS values for the myocardium immediately adjacent to the AR or BZ were analyzed for six sheep in each group. FS in the BZ was attenuated with ischemia and improved during reperfusion in both groups. There was a trend toward improved FS in the BZ during ischemia in sheep treated with acetaminophen ($P = 0.11$) but no significant differences at any other time point (Fig. 7).

**DISCUSSION**

Our investigation involved two different in vivo models of collateral-deficient myocardial ischemia and reperfusion that reliably simulate reperfusion therapy for AMI. Our results support the conclusion that in the setting of AMI, acetaminophen has a neutral effect on infarct size, myocardial blood flow, myocyte apoptosis, hemodynamics, and ventricular function.

We demonstrated a small but statistically significant increase in cardiac output and mean arterial pressure after acetaminophen was administered in sheep. This effect was abolished during the ischemia and reperfusion time periods. No difference in any other hemodynamic parameter was detected at any other time point in sheep. Similar inotropic effects could not be demonstrated in the rabbit. Using sonomicrometry, we also...
demonstrated a nonsignificant trend toward improvement in BZ contraction during ischemia but not immediately after drug delivery before ischemia or after reperfusion in sheep. The small and short-lived changes in cardiac output and mean arterial pressure in sheep and the lack of any hemodynamic effect in rabbits suggest that acetaminophen may have, at most, an exceedingly mild positive inotropic effect. These results are similar to those reported by Merrill et al. (27) in the dog. Merrill and colleagues (25, 26) have demonstrated that acetaminophen has antioxidant properties in the myocardium; however, little else is known of the effects of acetaminophen on myocardial function. Because we did not hypothesize that acetaminophen would have an effect on myocardial performance beyond myocardial salvage, our experiment was not designed to assess mechanistic questions regarding acetaminophen and myocardial contractility.

Since its introduction into western medicine in 1893, acetaminophen has been used primarily as an antipyretic and analgesic (23). It is a phenol that possesses antioxidant properties and can also inhibit prostaglandin synthesis through the inhibition of COX-3, an isoenzyme of cyclooxygenase (2). It should be noted that acetaminophen is a weak inhibitor of COX-1 and COX-2 cyclooxygenases, which are the predominant isoforms inhibited by the anti-inflammatory medications recently discovered to increase the risk of stroke and myocardial infarction (2, 15). The primary mechanism by which acetaminophen exerts its antipyretic and analgesic effects has yet to be definitively elucidated.

Over the last decade, off-label uses of acetaminophen for purposes other than pain relief have been reported. In human and canine studies of renal function, acetaminophen has been shown to reduce prostaglandin synthesis, renal blood flow, and glomerular filtration (4, 10). In a gastric model of ischemia-reperfusion, acetaminophen has been shown to preserve gastric mucosa by blocking hydroxyl radical-induced cellular damage (29). Acetaminophen has also been shown to have antioxidative effects in the myocardium of several mammalian species (25, 27). This antioxidant property has lead to the hypothesis that acetaminophen might have cardioprotective properties by preventing oxygen radical-induced reperfusion injury.

Recently, there have been conflicting reports regarding the effects of acetaminophen on ventricular function and myocardial salvage in the setting of reperfused AMI (6, 16, 27). Given the wide use of acetaminophen as an over-the-counter analgesic and antipyretic, the prevalence of undetected coronary disease, and the increasing use of reperfusion therapy to treat AMI, it is important to establish the safety and adjunctive benefits of the drug in this critically ill group of patients.

Data supporting cardioprotective effects of acetaminophen primarily come from the work of Merrill and colleagues (12, 13, 24–27, 31). In an isolated guinea pig heart model of myocardial ischemia and reperfusion, these investigators reported that acetaminophen-treated animals had preserved ventricular function during reperfusion compared with controls (25). Recently, Merrill and colleagues reported a 22% reduction in infarct size in an intact canine model of ischemia and reperfusion (27). In contrast to these findings, Kloner and colleagues (6, 16) have reported a neutral effect of acetaminophen on infarct size, regional myocardial blood flow, and ischemic preconditioning. This work was performed in rabbit and rat models of myocardial ischemia and reperfusion.

The differences in the animal models employed in the above cited studies may explain the inconsistent results reported by the two groups. Merrill’s use of the intact collateral-rich dog heart may be less clinically relevant than the intact small animal collateral-deficient models used by Kloner’s group. The dog, unlike humans, pigs, rabbits, and sheep, has extensive preformed epicardial collaterals that prevent absolute ischemia within the AR (9, 21, 22). The work of Kloner’s group is limited by the exclusive use of small animal models that are generally less tolerant of ischemia than large animal preparations (17). To address these limitations, we utilized intact large (sheep) and small (rabbit) animal collateral deficient models that closely replicate the clinical scenario of reperfused AMI.

Our results are, generally, more consistent with the findings reported by Kloner and colleagues. Possible explanations for the differing results of Merrill and colleagues may be related to dosage and the timing of acetaminophen administration. Hale and Kloner (16) showed no effect on infarct size with the use of a dose of 75 mg/kg, administered either before or during ischemia in rabbits. In the lone in vivo study showing a reduction in infarct size, Merrill et al. used a dose of 15 mg/kg of acetaminophen administered just before the onset of 60 min of ischemia and another 15 mg/kg dose after 90 min of reperfusion in dogs. The authors speculated that myocardial salvage effects of acetaminophen were related to its antioxidant properties, because acetaminophen-treated animals had significantly decreased circulating plasma concentrations of peroxynitrite during ischemia and reperfusion (27). We used a dose of 15 mg/kg in rabbits because it is the maximum-recommended single dose of acetaminophen. In the sheep we used 75 mg/kg, which is, depending on the animal’s size, 60% to 80% of the maximum daily human dose of 4 g. We chose this dose in an attempt to maximize the cardioprotective benefit while at the same time minimizing the potential for toxicity. This dose was also chosen to maximize the clinical relevance of the experiment by employing a single dose that would be considered clinically safe for use in patients. Finally, a higher dose was used in the sheep experiments because an assessment of myocardial perfusion was planned, and previous studies (24) had demonstrated a modest, but significant, coronary vasoconstrictive effect of the drug.

In this experiment, the drug was always infused intravenously 30 min before coronary artery occlusion. It is possible that the additional dose given by Merrill’s group after reperfusion contributed to the difference in results; however, the relatively long half-life of acetaminophen [150 min (32)] and the relatively large doses used in all the studies reported to date argue against this explanation.

We also examined the effect of acetaminophen on coronary blood flow. It is known that prostaglandins are released from ischemic myocardium and contribute to myocardial protection during reperfusion by increasing coronary blood flow (3, 33). This cardioprotective role of prostaglandins is not insignificant. It has been demonstrated in an ischemia-reperfusion model that mice lacking prostaglandin receptors have an increased myocardial infarct size compared with wild-type mice (36). Acetaminophen, which can inhibit prostaglandin synthesis, has been shown to modestly but significantly increase coronary vascular resistance and decrease coronary perfusion at doses at the upper limits of the human therapeutic range (24). Therefore, it was possible that high doses of the drug could signif-
significantly attenuate coronary blood flow and increase infarct size. This was not found to be the case in our study, because the results of our regional blood flow study showed that acetaminophen had no adverse or salutary effects on coronary blood flow in the AR at any time point in the study (Fig. 6).

In the current study, acetaminophen was dissolved in a 10% ethanol solution for intravenous administration. It has been shown that acute ethanol exposure does not affect infarct size after myocardial ischemia and reperfusion in a rabbit model, similar to the one used in this study (1). Also, it has been shown that short-term administration of alcohol had no effect on ventricular function after myocardial ischemia and reperfusion in a conscious canine model (35). Nevertheless, this is another difference between the current study and the work showing the cardioprotective benefits of acetaminophen, which used a physiological salt solution as the vehicle for acetaminophen. Therefore, it is possible that the effect of ethanol on the myocardium or its interaction with acetaminophen could mask potential cardioprotective effects from acetaminophen.

In summary, the current report adds to a small but growing body of data on the effects of acetaminophen in the setting of a reperfused AMI. In both large and small animal models of myocardial ischemia and reperfusion, we have shown that acetaminophen has a neutral effect on hemodynamics, heart rate, myocyte apoptosis, and myocardial infarct size. These results are both in concordance and in conflict with existing data in the literature. Despite the discrepancies between the studies published to date, none have demonstrated any harmful effects of the drug during myocardial ischemia or reperfusion. The recent awareness that COX-2 inhibitors increase the risk of effects of the drug during myocardial ischemia or reperfusion.

REFERENCES


