Acetaminophen and myocardial infarction in dogs

Gary F. Merrill, Tyler H. Rork, Norell M. Spiler, and Roseli Golfetti

Department of Cell Biology and Neurosciences, Division of Life Sciences, Rutgers University, Piscataway, New Jersey 08854

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Merrill, Gary F., Tyler H. Rork, Norell M. Spiler, and Roseli Golfetti. Acetaminophen and myocardial infarction in dogs. Am J Physiol Heart Circ Physiol 287: H1913–H1920, 2004. First published July 15, 2004; doi:10.1152/ajpheart.00565.2004.—The hypothesis that acetaminophen can reduce necrosis during myocardial infarction was tested in male dogs. Two groups were studied: vehicle- (n = 10) and acetaminophen-treated (n = 10) dogs. All dogs were obtained from the same vendor, and there were no significant differences in their ages (18 ± 2 mo), weights (24 ± 1 kg), or housing conditions. Selected physiological data, e.g., coronary blood flow, nonspecific collateral flow, epicardial temperature, heart rate, systemic mean arterial pressure, left ventricular developed pressure, the maximal first derivative of left ventricular developed pressure, blood gases, and pH, were collected at baseline and during regional myocardial ischemia and reperfusion. There were no significant differences in coronary blood flow, nonspecific collateral flow, epicardial temperature, heart rate, systemic mean arterial pressure, or blood gases and pH between the two groups at any of the three time intervals, even though there was a trend toward improved function in the presence of acetaminophen. Infarct size, the main objective of the investigation, was markedly and significantly reduced by acetaminophen. For example, when expressed as a percentage of ventricular wet weight, infarct size was 8 ± 1 versus 3 ± 1% (P < 0.05) in vehicle- and acetaminophen-treated hearts, respectively. When infarct size was expressed as percentage of the area at risk, it was 35 ± 3 versus 13 ± 2% (P < 0.05) in vehicle- and acetaminophen-treated groups, respectively. When area at risk was expressed as percentage of total ventricular mass, there were no differences in the two groups. Results reveal that the recently reported cardioprotective properties of acetaminophen in vitro can now be extended to the in vivo arena. They suggest that it is necessary to add acetaminophen to the growing list of pharmaceuticals that possess cardioprotective efficacy in mammals.

canine myocardium; heart disease; area at risk; electron microscopy

As science and medicine enter a new millennium, the century-old notion of the limited therapeutic actions of acetaminophen, i.e., relief of pain and reduction of fever, might soon be outdated. Merrill et al. (28, 29, 31, 32) reported that acetaminophen possesses cardioprotective properties in the postschismic, reperfused myocardium. Golfetti et al. (11, 12) showed that this is true even if acetaminophen is administered chronically or at the onset of reflow. Boutaud et al. (4) found concentration-dependent decrements in the production of prostacyclin when acetaminophen was used to block prostaglandin H synthase. Chou and Greenspan (7) reported that acetaminophen, by attenuating the activity of myeloperoxidase, significantly attenuated oxidation of low-density lipoprotein (LDL) in macrophages. Intramural oxidation of lipids and conversion of macrophages to foam cells in the same location are integral to intraluminal vascular disease (37, 38). Brennan et al. (5) recently reported that myeloperoxidase is a more sensitive and reliable predictor of future cardiac events in humans, including death, than other currently used indicators. Other as-yet-unpublished investigations are exploring the effects of acetaminophen on diet and vascular atherogenesis, spread of necrosis and apoptosis postmyocardial infarction, and procedurally induced myocardial infarction. The rationale for undertaking the current investigation was the following: 1) there are few in vivo studies describing the cardiovascular effects of acetaminophen, 2) there are no in vivo reports of acetaminophen efficacy during myocardial infarction (8, 15), and 3) there is a need for much more work in this arena in general. Time and continued experimentation will reveal the potential utility of acetaminophen to the treatment of cardiovascular disease in humans.

Methods

Animals. We performed all experiments in male dogs bred for research weighing 24 ± 1 kg and averaging 18 ± 2 mo. We housed the dogs in American Association for Accreditation of Laboratory Animal Care-accredited facilities where room temperature, humidity, and lighting were controlled. We fed the dogs a daily ration of Purina Dog Chow and provided access to water ad libitum. We allowed the dogs several days to acclimate to their new housing conditions and fasted them for about 24 h before the day of experimentation (water provided ad libitum). We obtained institutional review and approval before initiating the experiments.

Surgical preparation and instrumentation. On the day of experimentation we weighed the dogs and anesthetized them with pentobarbital sodium (30 mg/kg iv). We clipped hair from the inguinal region, the chest, and on the fore- and hindlimbs in the vivarium. We then transported the dogs to the experimental laboratory where they were intubated and ventilated on room air supplemented with 100% oxygen (Harvard Respirator, Harvard Apparatus; Millis, MA). We isolated and cannulated the right femoral artery and vein [polyethyl- ene (PE)-240 catheters filled with 0.9% NaCl solution], and we used the artery to monitor systemic arterial blood pressure (P\textsubscript{a}, pulsatile and mean) and the vein to administer supplemental anesthesia, heparin, acetaminophen, and vehicle. A left-sided thoracotomy was performed, and lobes of the left lung were gently retracted and the pericardium incised. A pericardial sling was made, and a 1.0-cm segment of the left anterior descending coronary artery (LAD) was isolated just beyond the third or fourth major lateral branches. A shunt was then constructed, as we have previously reported (9, 27, 30), between the LAD and the cannulated left subclavian artery. Coronary blood flow was monitored with a fiber optic-flow-probe (Statham P23ID, Oxnard, CA). The cost of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
(LAD flow) through the shunt was continuously measured ultrasonically (model T206 flowmeter, no. 4N69 extracorporeal, in-line flow probe, Transonic Systems; Ithaca, NY) thus enabling us to determine the state of LAD blood flow throughout the experiment. Coronary perfusion pressure was measured at the tip of the LAD cannula. We used a modification of the methods of Manor et al. (26) and Scheel et al. (40) to estimate nonspecific coronary collateral blood flow. To do this, the subclavian-to-LAD shunt was constructed of two segments of catheter in series (with the flow probe interposed between them). One segment, of 15-cm length of PE-240 tubing, was inserted into the isolated LAD. The other segment was implanted in the isolated subclavian artery. By briefly occluding the subclavian segment, disconnecting the LAD segment, and exposing it to ambient pressure at the level of the heart, we could collect retrograde coronary blood flow into preweighed vials. Such timed collections of retrograde blood flow, as reported by Manor et al. (26) and Scheel et al. (40), yield a reliable estimate of nonspecific, coronary collateral blood flow in the dog heart.

A saline-filled, short, large-bore catheter was then placed in the left ventricular chamber. This was used to determine left ventricular developed pressure (LVPD) and its differentiation (±dP/dt max). Subsequently, dogs were heparinized (250 U/kg plus supplements iv), and a standard limb lead electrocardiogram was attached and used to determine cardiac rate. Core body temperature (rectal probe) and epicardial surface temperature (Physitemp, model BAT-12; Clifton, NJ) were monitored continuously and maintained by elevating room temperature and by using heating blankets, plastic wrap (to close the opened thoracic cavity), and heat lamps. Dogs were then allowed time for monitored variables to achieve the steady state.

**Monitored variables and data acquisition.** Monitored variables included the following: core and epicardial surface temperatures (°C), coronary blood flow (in ml/min • 100 g-1), ventilatory frequency (in cycles/min), tidal volume (in ml), end-tidal CO2 (percent expired gases) (model NB-P75, Nellcor Puritan Bennett capnograph; Pleasanton, CA), oxyhemoglobin saturation (SaO2, in %, capnograph, model NB-P75), blood gases (Po2, Pco2, in mmHg), pH (in units) (model 248 blood gases/pH analyzer, Chiron Diagnostics; West Haven, CT), systemic mean arterial pressure (Psa, mmHg), LVPD (mmHg), and its ±dP/dt max, (in mmHg/s), heart rate (HR, cycles/min), and the ECG. The cardiovascular variables were monitored on a CB Sciences data acquisition system (model 214, iWorx; Dover, NH) in series with a computer running Labscribe software (version 6.0, CB Sciences, Dover, NH).

**Experimental protocol.** Two groups of dogs were studied. One group was treated with vehicle (acetaminophen solvent, 0.9% NaCl solution, n = 10) and the other treated with acetaminophen (total dose, 30 mg/kg iv, n = 10). Two bolus injections of acetaminophen were made, one just before the onset of ischemia (375 mg iv, i.e., 15 mg/kg) and the other after 90 min of reperfusion (375 mg iv, i.e., 15 mg/kg). A total dose of 750 mg acetaminophen was administered. Supplemental anesthesia was administered as needed. Once monitored variables were in the steady state, baseline data were collected (control, preischemia), and the LAD was occluded for 60 min. Subsequently, the LAD occlusion was released, and the ischemic myocardium was reperfused for 180 min. Core and epicardial temperatures, as well as coronary blood flow, respiratory variables, heart rate, ECG, Psa, LVPD, and ±dP/dt max were monitored continuously during the 4-h period of ischemia and reperfusion, as well as during the period preceding attainment of the steady state. Data for all other monitored variables were collected intermittently (e.g., retrograde coronary blood flow).

**Determining infarct size.** Standard procedures were used to estimate the location and extent of infarcted tissue. After the experimental protocol had been completed, dogs were euthanized, and hearts were rapidly excised and placed in warmed saline (37°C). All nonventricular tissue was removed, and ventricular wet weights were obtained. The aorta was cannulated (taking care to avoid penetrating the aortic valve), and hearts were transferred to a perfusion apparatus. Warmed (37°C) dyes were simultaneously perfused at physiological coronary perfusion pressure [triphenyltetrazolium chloride (TTC) 1.0%, into the cannulated LAD; Evans blue dye, 2.0%, into the aorta]. Both dyes were made fresh daily in phosphate buffer-dextrose solution that had been neutralized to pH 7.42 ± 0.02. After perfusion of dyes, hearts were placed in warmed saline (37°C) for 20 min for fixation. Subsequently, hearts were reperfused with formalin, removed from the perfusion apparatus, and stored in formalin at room temperature for about 48 h.

After 48 h in formalin, eight ventricular slices of near-uniform thickness (8–10 mm) were cut perpendicular to the long axis from base to apex. Each slice was weighed and placed under transparency film (apical then basal surfaces in contact with the film). Three areas of tissue were carefully traced onto the transparency: 1) viable tissue outside the area at risk that was stained by Evans blue dye, 2) viable tissue inside the area at risk that was stained brick red by TTC, and 3) necrotic tissue inside the area at risk that was not stained by TTC but rather was pale or colorless (infarcted tissue). After color-coded delineation, the areas of each region were estimated using compensating polar planimetry (model 1810-L30A, Dietzgen). The two surfaces for each slice were averaged, and the mass of each area was calculated. Infarct size was expressed as the estimated ventricular mass (in g) of necrotic tissue in each slice. It was also expressed as a percentage of total ventricular mass and as percentage of the LAD-perfused area at risk.

**Myofibrillar ultrastructure.** In two additional dogs (vehicle- and acetaminophen-treated dogs), we examined the myofibrillar ultrastructure using electronmicroscopy. At the end of the ischemia-reperfusion protocol, hearts were extracted and perfused (at physiological coronary perfusion pressure) with Karnovsky’s fixative. Full-thickness (transmural) tissue samples were collected from the nonischemic and ischemic zones of the left ventricular free wall. The blocks of tissue were postfixed with 1% osmium tetroxide, followed by dehydration in graded ethanol. Samples were embedded in Epon-Araldite cocktail, sectioned with a diamond knife ultramicrotome (model LKB-2088; LKB), and viewed with an electron microscope (model JEM-100CXII, JOEL) using standard methods. Electronmicroscopic images (n = 20 each from vehicle- and acetaminophen-treated samples) were visually inspected for the appearance of swollen mitochondria (a key indicator) and other signs of tissue damage. Other than visual inspection, no other objective scoring system was used to evaluate the images.

**Peroxyanitrle and antioxidant properties of acetaminophen.** Coronary venous plasma samples (0.5 ml each) were obtained at baseline, at about 60 min ischemia, and at about 180 min reperfusion. Using methods previously reported from this laboratory (29, 31), we evaluated the effects of acetaminophen on the production of blue light (chemiluminescence, i.e., peroxyanitrite-mediated oxidation of luminal) during these three experimental conditions.

**Statistical analysis.** The experimental design was determined a priori. Analysis of variance for repeated measures was used to make comparisons across time within either group, e.g., changes in HR, LVPD, etc., caused by ischemia and reperfusion. Student’s t-test for unpaired replicates (assuming unequal variance) was used to compare infarct size between the two groups. All data were expressed as means ± SE. Statistically significant differences were established at P < 0.05.

**RESULTS**

**General characteristics of dogs and ventricles.** No significant differences existed in the two groups of dogs (e.g., age, breeding conditions). Their average age was 18 ± 2 mo, and their average weight was 24 ± 1 kg. Ventricular wet weights are shown in Table 1 and did not vary between the two groups. Of the eight slices evaluated per heart, neither the mass of...
periods (i.e., baseline, 60 min ischemia, 180 min reperfusion) restored. For example, heart rates in the three experimental groups in any of the several hemodynamic variables monitored did not differ significantly between the two treatment groups. The area at risk was slightly more than 20% of the ventricular wet weight per heart (Table 1).

Blood gases, pH, and other metabolic variables. Partial pressures of oxygen and carbon dioxide did not vary significantly between the two groups during any of the three time intervals. The saturation of hemoglobin by oxygen nor the expired end-tidal carbon dioxide concentration did not differ between groups. For example, during the baseline period, PO₂ in acetaminophen- and vehicle-treated dogs, respectively, was 137 ± 11 mmHg versus 123 ± 9 mmHg. During the same time period, SaO₂ values in the two groups were 99 ± 0.2% versus 98 ± 0.5%. These did not change significantly during ischemia or reperfusion, in part, because ventilation was supplemented with 100% oxygen. Other indicators of ventilation, e.g., PCO₂, CO₂ content of arterial blood, HCO₃⁻, pH, and base excess, did not differ significantly between dogs in the two groups (Table 2). Neither were there significant differences in ventilatory frequency, tidal volume, core body, and epicardial surface temperatures.

Hemodynamics and ventricular function. There were no statistically significant differences between the two treatment groups in any of the several hemodynamic variables monitored. For example, heart rates in the three experimental periods (i.e., baseline, 60 min ischemia, 180 min reperfusion) ranged from 145 ± 5 to 168 ± 6 cycles/min, and mean systemic arterial blood pressure ranged from 110 ± 7 to 119 ± 6 mmHg during the same time intervals (there were no significant differences) (Table 3).

Left ventricular mechanical function did not differ significantly between the two groups at any time during the experiment. Both LVDP and +dP/dₜmax declined significantly (P < 0.05) from baseline to the end of reperfusion (−dP/dₜmax also showed tendencies toward declining, but these did not become statistically significant). Overall, there was a trend toward acetaminophen-treated hearts developing greater force and maintaining greater ventricular function than vehicle-treated hearts, but these trends did not achieve statistical significance (Table 3).

ECG and heart rate. Hearts were not electrically paced in this investigation, nor did heart rate, in either group, differ significantly across time during the experiment. Electrocardiographically, a variety of ventricular ectopic beats could be identified, e.g., ventricular premature beats, ventricular salvos, and ventricular tachycardia. Nonsustained ventricular tachycardia occurred regularly during reperfusion in vehicle-treated dogs (e.g., in 4 of 10 dogs) but was less frequent in acetaminophen-treated dogs (1 of 10 dogs). Whereas the objective of this experiment was not to assess the antiarrhythmic potential of acetaminophen, in general it appeared that acetaminophen-treated dogs were more stable electrically than vehicle-treated dogs.

Coronary and collateral blood flow. There were no statistically significant differences in blood flow through the cannulated LAD in the two groups at any time in this experiment. Flow through the LAD shunt fell to zero in both groups upon occlusion and remained there during the 60-min period of ischemia. Upon reperfusion LAD flow rate in both groups increased above corresponding baseline values (transient reactive hyperemia) but returned to baseline by 180 min (Fig. 1). There were no significant differences in coronary perfusion pressure and calculated coronary vascular resistance between the two groups at any timed interval (Table 4). Nonspecific collateral blood flow was also similar in the two groups at baseline and during ischemia and reperfusion (Table 4).

Infarct size. Ventricular wet weights were 166 ± 8 and 171 ± 9 g in vehicle- versus acetaminophen-treated hearts, respectively, and did not differ significantly. The areas at risk did not differ between the two treatments (Fig. 2), even though the actual size of the infarcts was significantly smaller in acetaminophen-treated hearts [22 ± 3 vs. 9 ± 2 g (P < 0.05)]. When the size of the area at risk was expressed as a percentage of the entire ventricular myocardium, there were no significant differences between the two groups, i.e., about 20–21% in each. Infarct size, expressed as a percentage of the area at risk in vehicle-treated hearts, was 35 ± 3%; however, in acetaminophen-treated dogs it was 13 ± 2% (P < 0.05) (Table 1). When infarct size was expressed as a percentage of the entire

Table 1. Dimensions of dogs, ventricles, and ventricular areas at risk of myocardial infarction during ischemia-reperfusion injury in the absence (vehicle-treated) and presence of acetaminophen

<table>
<thead>
<tr>
<th></th>
<th>Dog Weight, kg</th>
<th>VWW, g</th>
<th>AAR, % VWW</th>
<th>Infarct Size, %AAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle treatment</td>
<td>23.8±0.7</td>
<td>166±8</td>
<td>21±2</td>
<td>35±3</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>25.0±0.7</td>
<td>171±9</td>
<td>20±4</td>
<td>13±2*</td>
</tr>
<tr>
<td>Combined</td>
<td>24.5±0.7</td>
<td>168±8</td>
<td>21±5</td>
<td>24±2</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 10 dogs. VWW, ventricular wet weight; AAR, area at risk. *P < 0.05 relative to corresponding vehicle-treated value.

Table 2. Blood gases, pH, and other indexes of respiratory/metabolic status of dogs during regional myocardial ischemia-reperfusion injury in the absence (vehicle) and presence of acetaminophen

<table>
<thead>
<tr>
<th></th>
<th>PO₂, mmHg</th>
<th>SaO₂, %</th>
<th>PCO₂, mmHg</th>
<th>[CO₂], mmol</th>
<th>EtCO₂, %</th>
<th>pH, units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>123±9</td>
<td>98±0.5</td>
<td>32±1</td>
<td>20±0.3</td>
<td>3.1±0.2</td>
<td>7.40±0.02</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>137±11</td>
<td>99±0.2</td>
<td>29±2</td>
<td>20±0.5</td>
<td>2.6±0.2</td>
<td>7.44±0.02</td>
</tr>
<tr>
<td>60 min Ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>142±6</td>
<td>96±0.6</td>
<td>31±1</td>
<td>20±0.5</td>
<td>2.7±0.2</td>
<td>7.40±0.02</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>137±15</td>
<td>99±0.5</td>
<td>29±1</td>
<td>19±0.5</td>
<td>2.7±0.2</td>
<td>7.43±0.01</td>
</tr>
<tr>
<td>180 min Reperfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>129±14</td>
<td>96±1.0</td>
<td>25±2</td>
<td>17±1.4</td>
<td>2.7±0.2</td>
<td>7.42±0.01</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>131±16</td>
<td>97±1.0</td>
<td>25±2</td>
<td>18±0.6</td>
<td>2.8±0.2</td>
<td>7.41±0.02</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 10 dogs. PO₂, partial pressure of O₂ in arterial blood; SaO₂, oxyhemoglobin saturation of arterial blood; PCO₂, partial pressure of CO₂ in arterial blood; [CO₂], content of CO₂ in arterial blood; EtCO₂, end-tidal CO₂; pH, acidity/alkalinity of arterial blood.
myocardium, the difference was equally significant, i.e., $8 \pm 1\%$ vs. $3 \pm 1\%$ ($P < 0.05$) (Figs. 3 and 4).

Myofibrillar ultrastructure. Ultrastructurally, myofibrils of vehicle-treated hearts displayed more damage than those of acetaminophen-treated hearts. This is true of samples taken in both the nonischemic and ischemic zones. For example, in the area at risk (ischemic zone) there was general evidence of sarclemmal and nuclear damage, less well-defined sarcosomes, occasional contraction bands, and marked swelling of mitochondria in vehicle-treated samples. Such evidence of damage was harder to find in images taken from acetaminophen-treated samples. For example, the mitochondria were less swollen, and the cristae were more densely packed in the presence of acetaminophen (Fig. 5).

Peroxynitrite and acetaminophen. There were no significant differences in circulating plasma concentrations of peroxynitrite under baseline, control conditions in the two groups. Conversely, acetaminophen significantly decreased peroxynitrite during both ischemia and reperfusion (Fig. 6).

**DISCUSSION**

The list of pharmacological agents shown to possess cardioprotective efficacy (as measured by reduction of necrosis and infarct size) is growing and includes scavengers of oxygen and/or nitrogen free radicals (16, 20), antiapoptotic agents (10, 44), calcium channel antagonists (17, 43), adenosine and its analogs (36), inhibitors of the complement system (2, 6, 39), nitric oxide (19, 23), inhibitors of neutrophils and/or macrophages (21, 22), renin-angiotensin inhibitors (18, 41), endothelin antagonists (13, 14), inhibitors of the Na/H exchanger (25, 33), and antioxidants (1, 42).

To date we have found evidence in vitro that acetaminophen protects cardiac function and preserves myocardial tissue during low-flow, global myocardial ischemia and reperfusion in Langendorff-perfused guinea pig hearts. This is true whether the agent is administered before, during, or after ischemia, as well as when it is administered chronically (11, 12, 29, 31, 32). Acetaminophen appears to have similar cardioprotective properties during hypoxia-reoxygenation (unpublished observations). Of course the crystalloid-perfused Langendorff rodent-heart preparation is far removed from the neurohumorally intact, afterloaded, blood-perfused whole heart under in situ conditions. Thus there is a need to extend such in vitro experiments to the in vivo arena.

**Dose of acetaminophen.** We selected the 30 mg/kg dose of acetaminophen for several reasons. First, we wanted to be consistent with others who are currently using acetaminophen in animal experimentation. For example, at a symposium focusing on the actions of acetaminophen in animals, investigators reported using doses of 15 mg/kg (rabbits), 60 mg/kg (mice), ~60 mg/kg (humans), and 10–1,000 mg/kg (rats) (Tylenol Research Symposium, September 29, 2003, Fort...
Washington, PA, personal communications). None of these doses was reported to have toxic effects. Second, we wanted to avoid the prospects of either hepatic or renal toxicity. The maximum recommended daily dose of Extra Strength Tylenol, for example, is about 60 mg/kg per day. This dose is nontoxic in humans. Our dose of 30 mg/kg is half this and would not be expected to produce any toxic effects in the dogs. However, the surgical interventions, i.e., opening the chest, are serious and might be expected to complicate the actions of acetaminophen. If this happened, such changes were not revealed in the monitored variables (e.g., renal-mediated toxic effects might be expected to change systemic arterial blood pressure, which did not happen). Finally, we wanted to maximize our chances of seeing an effect of the drug in vivo. By administering half of the total dose before the onset of ischemia and the other half after 90 min of reperfusion, we assumed that circulating plasma concentrations would be near optimal during ischemia and reperfusion. This timing is consistent with the reports of Jolly et al. (20) and Bolli et al. (3), and with knowledge that the early minutes of reperfusion are critical to the production-release of damaging oxidants (3, 27), some of which have been shown to be attenuated by acetaminophen (27, 32).

General mechanical, hemodynamic, and metabolic status of the dogs. The reason we monitored left ventricular function was to establish that the two groups of dogs were in a similar physiological state throughout the experiment. Measures of LVDP and ±dP/dt max enabled us to achieve that goal. A similar argument applies to the collection of general respiratory-metabolic data. The absence of differences in ventricular function and in the general respiratory-metabolic status of the dogs suggests that statistically significant variability in myocardial infarction cannot be explained on the basis of ventricular mechanics, metabolism, or respiration. Had, for example, oxygen saturation and the partial pressures of oxygen in vehicle-treated dogs been significantly reduced relative to acetaminophen-treated dogs, one might have argued a causative role for reduced myocardial oxygenation. Likewise, neither can differences be explained on the basis of metabolic hypercapnia-acidosis because all indexes of the production-metabolism of acid were similar in the two groups.

Heart rate and the ECG. We did not pace hearts electrically in this experiment. Nonetheless, there were no differences in heart rates between the two groups during any of the three periods of data collection. Therefore, changes in heart rate and their contributions to myocardial oxygen supply and demand cannot explain the differences in infarct size and necrosis. Moreover, investigating the potential antiarrhythmic actions of acetaminophen was not an objective of this study. However, during both ischemia and reperfusion, acetaminophen-treated hearts appeared much more stable, electrically, than vehicle-treated hearts. For example, nonsustained ventricular tachycardia occurred regularly in vehicle-treated dogs during reperfusion but was less evident in the presence of acetaminophen. In the isolated, perfused guinea pig heart, toxic doses of pentobarbital sodium were not as arrhythmogenic in the presence of acetaminophen (31). Thus an investigation of the potential antiarrhythmic qualities of acetaminophen, in an in vivo setting, should be conducted.

Coronary and collateral blood flow. Neither of these two variables differed between the two groups of dogs under any condition. The coronary vasculature was viable and responsive in both groups (e.g., brisk hyperemic flow responses upon release of 15-s periods of inflow occlusion; data not shown) and in a physiological state in all regards (e.g., control, baseline values that are consistent with previous reports from our
laboratory (27, 30) and from other laboratories (20, 26). Neither did nonspecific collateral coronary blood flow differ at any time interval in the two groups. Gathering data on collateral blood flow in this fashion did not interfere with the ischemia-reperfusion protocol as these samples were collected at the ends of ischemia (no LAD flow) and reperfusion (termination of the experiment). Additionally, the ~60 s needed to collect blood samples under baseline conditions is too short a period to cause preconditioning effects, which are only achieved by multiple, successive, longer-lasting periods of ischemia. Finally, if there had been any preconditioning of the myocardium, it would have occurred equally in the two groups of hearts and could not have biased the data.

Tissue injury, myocardial infarction, and acetaminophen. Efforts to improve our understanding of the actions of acetaminophen in the mammalian cardiovascular, and related systems, are gaining momentum. In this regard one of the first reports was that of Nakamoto et al. (35). They showed that acetaminophen protected against injury to the gastric mucosa caused by ischemia and reperfusion. Acetaminophen significantly reduced the area of mucosal erosions and simultaneously inhibited hydroxyl radical-induced elevations in lipid peroxides. We have shown that acetaminophen significantly reduces ventricular dysfunction caused by exogenously administered hydrogen peroxide (32) and that it attenuates the burst of hydroxyl radicals released in the early minutes of reperfusion following global myocardial ischemia (31). Chou and Greenspan (7) reported that a concentration of 0.25 mM acetaminophen significantly attenuates the actions of myeloperoxidase on the oxidation of LDL in macrophages. Intramural oxidation of LDL and conversion of macrophages to foam cells are integral to the process of luminal vascular atherogenesis and inflammation (37, 38). Brennan et al. (5) assessed the value of plasma concentrations of myeloperoxidase in the prognosis of cardiovascular events in patients presenting with chest pain. They concluded that “...a single initial measurement of plasma myeloperoxidase independently predicts the early risk of myocardial infarction, as well as the risk of major adverse cardiac events in the ensuing 30-day and 6-mo periods...” They included death as one of the cardiac events. In contrast to troponin T, creatine kinase MB isoform, and C-reactive protein, myeloperoxidase levels identified patients at risk of cardiac events in the absence of myocardial necrosis (5). Boutaud et al. (4) investigated the effects of acetaminophen on the activity of prostaglandin H synthase in endothelial cells of human umbilical vein extracts. In such tissues, prostacyclin
synthesis occurs predominantly in the endothelium and vascular smooth muscle cells of the vessel wall. Prostaglandin H synthase has both peroxidase and cyclooxygenase binding sites and is implicated in the pathogenesis of vascular disease. When the extracts of Boutaud et al. (4) were stimulated with IL-1α (using arachidonic acid as substrate), acetaminophen blocked the actions.

**Peroxy nitride and acetaminophen.** Technically, acetaminophen is not an nonsteroidal anti-inflammatory drug (i.e., its anti-inflammatory properties/potential in myocardial infarction have not been studied). We did not investigate its effects on cyclooxygenase activity in the present study. Thus we cannot draw conclusions about its ability to attenuate chemokines, cytokines, adhesion molecules, etc., etc. Mechanistically, acetaminophen-treated dogs showed a marked and significant attenuation of peroxynitride during ischemia and reperfusion. Peroxy nitride is an oxidant that is known to damage cell membranes and to alter the structure and function of macromolecules in biological systems. Thus it is tempting to speculate that its cardioprotective mechanism is via antioxidation.

This does not necessarily mean, however, that acetaminophen is an active oxygen species scavenger (i.e., perhaps reduction of peroxynitride is secondary to an acetaminophen-mediated reduction in the severity of ischemic injury). Nonetheless, these results obtained in vivo are consistent with our previous experience in vitro (29, 31, 32) and with the recent reports of others (7, 35). In addition to its actions against peroxynitride, we have shown acetaminophen to be efficacious against hydroxyl radical (31) and hydrogen peroxide (29). The work of others implicates myeloperoxidase (7), cyclooxygenase (4), and other peroxides (35) as additional targets of acetaminophen. R. C. Gorman is using acetaminophen to check the spread of apoptosis-necrosis postmyocardial infarction in rabbits and sheep. His early results are encouraging and suggest a positive trend for the drug (personal communications).

Contrary to our findings reported here, there are two neutral reports in the current literature worth mentioning. Dai and Klomer (8) and Hale and Klomer (15) reported no benefit of acetaminophen on infarct size in the rat and rabbit myocardium, respectively. Unfortunately, in the first study, there is no mention of myocardial blood flow (rats), and in the second study (rabbit) the period of ischemia (30 min) has been considered too short to produce substantial infarction in most mammalian species studied. Also, there is no defense by these authors of their choice of alcohol as the solvent for acetaminophen. Alcohol has well-known direct and indirect effects both on the mammalian myocardium (e.g., positive and negative chronotropy, inotropy, and dromotropy have been reported) and on the coronary circulation. Acetaminophen is easily solubilized in physiological salt solutions. This eliminates the possibilities of unwanted effects of organic solvents that mask and/or reverse the actions of solute.

**Summary and conclusions.** In the current investigation, the sites of isolation and ligation of the LAD were highly reproducible from dog to dog. Consistently, the LAD-occluded area at risk was slightly more than 20% of the total ventricular mass analyzed in both groups of hearts. Thus dog-to-dog variability in the size of the area at risk cannot explain the significant difference in size of infarcted tissue in the two groups. During regional myocardial ischemia and reperfusion in dogs, acetaminophen markedly and significantly reduced tissue necrosis and infarct size. Results extend our recent work in vitro (11, 12, 29, 31, 32) to the in vivo arena and reveal salutary effects of acetaminophen in the neurohumorally intact, blood-perfused tissue environment of the canine myocardium. Results suggest that acetaminophen is among the most efficacious of the cardioprotective agents discovered to date (44). However, we do have a note of caution. Metabolism of acetaminophen by dogs is different from that in humans. Therefore, the results obtained at the doses used might only be applicable to dogs and not to humans. More work is needed to reveal the potential salutary effects of this agent in other tissues and organ systems under other physiological and/or pathophysiological conditions (28).

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**REFERENCES.**


