Acetaminophen and Myocardial Infarction in Dogs

by

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Funded by McNeil Consumer and Specialty Pharmaceuticals, Fort Washington, PA, and by Johnson & Johnson, COSAT, New Brunswick, NJ

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ABSTRACT

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). All dogs were obtained from the same vendor and there were no significant differences in their ages (18±2 months), 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, ±dP/dt\textsubscript{max}, 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 towards 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 per cent of ventricular wet weight, infarct size was 8±1 vs 3±1 (P<0.05) in vehicle- and acetaminophen-treated hearts, respectively. When infarct size was expressed as per cent of the area at risk it was 35±3 vs 13±2 (P<0.05) in vehicle and acetaminophen treated groups, respectively. When area at risk was expressed as per cent 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.

Key words: canine myocardium, heart disease, area at risk, electronmicroscopy
INTRODUCTION

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notion of the limited therapeutic actions of acetaminophen, i.e. relief of pain and reduction of fever, might soon be outdated.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 post-ischemia, 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 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 acetaminophen’s effects on diet and vascular atherogenesis, spread of necrosis and apoptosis post-myocardial infarction, and procedurally-induced myocardial infarction.

The rationale for undertaking the current investigation was: 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 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 man.
METHODS

Animals---We performed all experiments in male dogs bred for research weighing 24±1kg and averaging 18±2 months. We housed the dogs in AAALAC 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 hours 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 using sodium pentobarbital (30 mg/kg, i.v.). 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 per cent oxygen (Harvard Respirator, Harvard Apparatus, Millis, MA). We isolated and cannulated the right femoral artery and vein (PE240 catheters filled with 0.9% NaCl solution), and used the artery to monitor systemic arterial blood pressure (Pā, 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 (LAD flow) through the shunt was continuously measured ultrasonically (model T206 flowmeter, #4N69 extracorporeal, in-line flow probe, Transonic Systems, Inc., 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, a 15 cm length of PE240 tubing, was inserted into the isolated LAD. The other segment was implanted in the isolated subclavian artery. By briefly occluding the subclavian segment, and 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 and its differentiation. Subsequently, dogs were heparinized (250U/kg plus supplements, i.v.), and a standard limb lead electrocardiogram was attached and used to determine heart 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 use of 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 (CBF, ml/min/100g), ventilatory frequency (Vf, cycles/min), tidal volume (Vt, ml), end tidal CO₂ (percent expired gases) (Nellcor Puritan Bennett capnograph, model NPB-75, Pleasanton, CA),
oxyhemoglobin saturation (per cent, SaO₂, capnograph, model NPB-75), blood gases (PO₂, PCO₂, mmHg) and pH (units) (Chiron Diagnostics, model 248 blood gases/pH analyzer, West Haven, CT), systemic mean arterial pressure (Pₐ, mmHg), left ventricular developed pressure (LVDP, mmHg), and its differentiation (±dP/dt max, mmHg/sec), heart rate (HR, cycles/min), and the electrocardiogram (ECG). The cardiovascular variables were monitored on a CB Sciences data acquisition system (iWorx model 214, 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, 30mg/kg i.v., n=10). Two bolus injections of acetaminophen were made, one just before the onset of ischemia (375 mg i.v., i.e. 15mg/kg), and the other after 90min of reperfusion (375 mg i.v., i.e. 15mg/kg). A total dose of 750mg 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, Pₐ, LVDP, and ±dP/dt max were monitored continuously during the four hour 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. Upon completion of the experimental protocol, dogs were euthanized and hearts were rapidly excised and placed in warmed saline (37°C). All non-ventricular 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; Evan’s 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 hours.

After 48 hours 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 Evan’s 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). Following 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 (grams) of necrotic tissue in each slice. It was also
expressed as per cent of total ventricular mass, and as per cent of the LAD-perfused area at risk.

**Myofibrillar ultrastructure**---In two additional dogs, vehicle- and acetaminophen-
treated, we examined the myofibrillar ultrastructure using electron microscopy. 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 non-ischemic 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, Sweden) and viewed with an electron microscope (model JEM-100CXII, JOEL) using standard methods. EM 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.

**Peroxynitrite and antioxidant properties of acetaminophen---**Coronary venous plasma samples (0.5ml each) were obtained at baseline, at about 60min ischemia, and at about 180min 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. peroxynitrite-mediated oxidation of luminol) 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, LVDP, etc. caused by ischemia and reperfusion. Students t-test for unpaired replicates (assuming unequal variance) was used to compare infarct size between the two groups. All data were expressed as means ± one standard error of the mean (s.e.m.). 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 months, 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 individual slices nor their summed weight varied between the two groups. The area at risk was slightly more than 20 per cent 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. Neither did the saturation of hemoglobin by oxygen, nor did the expired end tidal carbon dioxide concentration differ between groups. For example, during the baseline period, PO$_2$ in acetaminophen- and vehicle-treated dogs, respectively, was 137±11 vs 123±9 mmHg. During the same time period, SaO$_2$ values in the two groups were 99±0.2 vs 98±0.5 per cent. These did not change significantly during ischemia or reperfusion, in part, because ventilation was supplemented with 100% oxygen. Other indicators of ventilation, e.g. PCO$_2$, CO$_2$ content of arterial blood, HCO$_3^-$, 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, 60min ischemia, 180min 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/dt_{max} declined significantly (P<0.05) from baseline to the end of reperfusion (–dP/dt_{max} also showed tendencies toward declining, but these did not become statistically significant). Overall, there was a trend towards 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).

**Electrocardiogram 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 (VPBs), ventricular salvos (VS), and ventricular tachycardia (VT). 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). While 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 60min period of ischemia. Upon reperfusion LAD flowrate in both groups increased above corresponding baseline values (transient reactive hyperemia), but returned to baseline by
180min (Fig. 1). Nor were there 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- vs acetaminophen-treated hearts, respectively, and did not differ significantly. Neither did the areas at risk 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±2g (P<0.05)]. When expressing the size of the area at risk as a per cent 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 per cent 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 per cent of the entire myocardium the difference was equally significant, i.e. 8±1% vs 3±1% (P<0.05) (Figs. 3,4).

**Myofibrillar ultrastructure**---Ultrastructurally, myofibrils of vehicle-treated hearts displayed more damage than those of acetaminophen-treated hearts. This is true of samples taken from both the nonischemic and ischemic zones. For example, in the area at risk (ischemic zone) there was general evidence of sarcolemmal and nuclear damage, less well-defined sarcomeres, 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/nitrogen free radicals (16, 20), anti-apoptotic 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/macrophages (21, 22), renin-angiotensin inhibitors (18, 41), endothelin antagonists (13, 14), inhibitors of the sodium/hydrogen 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 30mg/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 acetaminophen’s actions in animals, investigators reported using doses of 15 mg/kg (rabbits), 60 mg/kg (mice), approximately 60 mg/kg (humans), and 10-1000 mg/kg (rats) (Tylenol Research Symposium, September 29, 2003, Fort Washington, PA, personal communications). None of these doses was
reported to have toxic effects. Secondly, 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 60mg/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 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 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 *etal* (20) and Bolli *etal* (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 for monitoring 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/dtmax enabled us to achieve that goal. A similar argument applies to 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 since all indices of the production/metabolism of acid were similar in the two groups.

**Heart rate and the electrocardiogram**---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 sodium pentobarbital 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 15sec 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 approximately 60 sec 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-month 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.

**Peroxynitrite and acetaminophen**—Technically, acetaminophen is not an NSAID (i.e. its anti-inflammatory properties/potential in myocardial infarction have not been studied). Nor did we investigate its effects on COX 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 peroxynitrite during ischemia and reperfusion. Peroxynitrite 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 ROS scavenger (i.e. perhaps reduction of peroxynitrite 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 peroxynitrite, 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. Gorman et al are using acetaminophen to check the spread of apoptosis/necrosis post-myocardial infarction in rabbits and sheep. Their early results are encouraging and suggest a positive trend for the drug (personal communications). Block prescribed 500mg acetaminophen twice daily to patients with carotid artery disease, and found after one year a 30-50 per cent reduction in blockage (personal communications).

Contrary to our findings reported here, there are two neutral reports in the current literature worth mentioning. Dai and Kloner (8) and Hale and Kloner (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 (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 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 per cent 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 amongst the most efficacious of the cardioprotective agents discovered to date (44). A note of caution, however. 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/pathophysiological conditions (28).
ACKNOWLEDGEMENTS

We acknowledge with gratitude the generous support of McNeil Consumer and Specialty Pharmaceuticals, Fort Washington, PA, and Johnson & Johnson COSAT, New Brunswick, NJ. We express appreciation to Mr. Brent Smith for editing the manuscript, and for his helpful writing suggestions. We also express our gratitude and appreciation to Mr. Valentin Starovoytov who prepared the electronmicroscopic images.

Experiments were conducted during the visit of Dr. Roseli Golfetti, State University of Sao Paulo at Campinas, Campinas, Brazil.
REFERENCES


FIGURE LEGENDS

Figure 1 LAD blood flow, measured ultrasonically, in vehicle-treated (black bars) and acetaminophen-treated hearts (gray bars). These histograms reflect data collected at the ends of baseline, ischemia, and reperfusion conditions. Data are presented as means ± 1 s.e.m. (n=10 each). Note absence of differences between the two groups.

Figure 2 Histograms of ventricular wet weights and areas at risk taken from vehicle-treated (black bars) and acetaminophen-treated hearts (gray bars). Note the absence of differences between the two groups.

Figure 3 Histograms of infarct size expressed as per cent of ventricular mass (left side) and area at risk (right side) in vehicle-treated (black bars) and acetaminophen-treated hearts (gray bars). Note the statistically significant differences in the presence of acetaminophen.

Figure 4 Actual images of infarcted tissue in vehicle- and acetaminophen-treated hearts (aided by computer graphics). Note the substantial decrease in infarcted tissue (white area) in the presence of acetaminophen.

Figure 5 Electronmicrographs of ischemic zone (area at risk, top panel) and nonischemic zone (bottom panel) in vehicle-treated (left image in each panel) and acetaminophen-treated tissue samples (right image in each panel). Arrows indicate tissue damage, including swollen
mitochondria and fragmented nucleus in vehicle-treated samples. Similar damage was not seen in acetaminophen-treated samples.

**Figure 6** Histogram of peroxynitrite-mediated chemiluminescence in vehicle- (black bars) and acetaminophen-treated (gray bars) hearts at baseline (left), after 60 min ischemia (middle), and after 180 min reperfusion (right). *, P<0.05 relative to corresponding bar in vehicle-treated hearts.
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>
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<th></th>
<th>dog weight (kg)</th>
<th>vww (g)</th>
<th>aar (% vww)</th>
<th>infarct size (% aar)</th>
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<td>Vehicle-treated</td>
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<td>21±2</td>
<td>35±3</td>
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<tr>
<td>Acetaminophen-treated</td>
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<td>171±9</td>
<td>20±4</td>
<td>13±2*</td>
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<tr>
<td>Combined</td>
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</tbody>
</table>

Data are means ± 1 s.e.m (n=10). vww, ventricular wet weight; aar, area at risk; *, P<0.05 relative to corresponding vehicle-treated value.
Table 2  Blood gases, pH, and other indices 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><a href="mmol">CO₂</a></th>
<th>EtCO₂(%)</th>
<th>pH(units)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>baseline</strong></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><strong>60 min ischemia</strong></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><strong>180 min reperfusion</strong></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 ± 1 s.e.m (n=10). PO₂, partial pressure of oxygen in arterial blood; SaO₂, oxyhemoglobin saturation of arterial blood; PCO₂, partial pressure of carbon dioxide in arterial blood; [CO₂], content of carbon dioxide in arterial blood; EtCO₂, end tidal carbon dioxide; pH, acidity/alkalinity of arterial blood.
<table>
<thead>
<tr>
<th></th>
<th>HR (cpm)</th>
<th>P(\text{a}) (mmHg)</th>
<th>LVDP (mmHg)</th>
<th>+dP/dt(_{\text{max}}) (mmHg/sec)</th>
<th>-dP/dt(_{\text{max}}) (mmHg/sec)</th>
<th>PRP (mmHg x cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vehicle</td>
<td>155±8</td>
<td>118±7</td>
<td>121±7</td>
<td>3141±247</td>
<td>2798±250</td>
<td>18540±1312</td>
</tr>
<tr>
<td>acetaminophen</td>
<td>145±5</td>
<td>119±6</td>
<td>129±9</td>
<td>3375±288</td>
<td>3215±300</td>
<td>18485±1625</td>
</tr>
<tr>
<td><strong>60 min ischemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vehicle</td>
<td>155±8</td>
<td>113±5</td>
<td>118±9</td>
<td>3195±205</td>
<td>2863±240</td>
<td>18087±1342</td>
</tr>
<tr>
<td>acetaminophen</td>
<td>152±5</td>
<td>119±4</td>
<td>128±8</td>
<td>3058±224</td>
<td>3105±243</td>
<td>19546±1362</td>
</tr>
<tr>
<td><strong>180 min reperfusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vehicle</td>
<td>168±6</td>
<td>110±7</td>
<td>114±8</td>
<td>2165±188*</td>
<td>2559±276</td>
<td>17393±1269</td>
</tr>
<tr>
<td>acetaminophen</td>
<td>159±5</td>
<td>117±6</td>
<td>116±6</td>
<td>2414±128*</td>
<td>2921±195</td>
<td>18648±1365</td>
</tr>
</tbody>
</table>

Data are means ± 1 s.e.m (n=10). HR, heart rate; cpm, cycles per minute; P\(\text{a}\), mean systemic arterial pressure; LVDP, left ventricular developed pressure; +dP/dt\(_{\text{max}}\), rate of development of left ventricular pressure; -dP/dt\(_{\text{max}}\), rate of decline of left ventricular pressure; PRP, pressure-rate product; *, P<0.05 relative to corresponding baseline data.
Table 4  Coronary circulatory variables in dogs during ischemia-reperfusion injury in the absence (vehicle-treated) and presence of acetaminophen.

<table>
<thead>
<tr>
<th></th>
<th>Baseline conditions</th>
<th>60min ischemia</th>
<th>180min reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle-treated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD flow (ml/min/100g)</td>
<td>78±8</td>
<td>0±0</td>
<td>72±11</td>
</tr>
<tr>
<td>LAD perfusion pressure (mmHg)</td>
<td>85±4</td>
<td>15±2</td>
<td>82±8</td>
</tr>
<tr>
<td>LAD resistance (mmHg/ml/min/100g)</td>
<td>1.2±0.6</td>
<td>0±0</td>
<td>1.5±0.9</td>
</tr>
<tr>
<td>ncc flow (ml/min/100g)</td>
<td>8±2</td>
<td>8±1</td>
<td>7±3</td>
</tr>
<tr>
<td><strong>Acetaminophen-treated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD flow (ml/min/100g)</td>
<td>69±11</td>
<td>0±0</td>
<td>72±9</td>
</tr>
<tr>
<td>LAD perfusion pressure (mmHg)</td>
<td>85±9</td>
<td>15±3</td>
<td>82±6</td>
</tr>
<tr>
<td>LAD resistance (mmHg/ml/min/100g)</td>
<td>1.4±0.8</td>
<td>0±0</td>
<td>1.3±0.7</td>
</tr>
<tr>
<td>ncc flow (ml/min/100g)</td>
<td>9±3</td>
<td>8±2</td>
<td>8±3</td>
</tr>
</tbody>
</table>

Data are means ± 1 s.e.m. (n=10).  LAD, left anterior descending coronary artery; ncc, nonspecific coronary collateral blood flow.
Figure 1

![Graph showing LAD Flow (ml/min/100g) during Baseline, Ischemia, and Reperfusion for vehicle and acetaminophen groups.](image)
Figure 2

ventricular wet weight                              area at risk

vehicle
acetaminophen

ventricular mass (grams)
Figure 3

The graph shows the infarct/ventricular mass and infarct/area at risk normalized (per cent) for vehicle and acetaminophen treatments. The asterisk (*) indicates a statistically significant difference with a P<0.05.
Figure 4

Vehicle-treated

Acetaminophen-treated
Figure 6

- Vehicle
- Acetaminophen

Chemiluminescence (cpm x 10^-7)

Baseline
Ischemia
Reperfusion

* P<0.05