Acetaminophen Is Cardioprotective Against H_2O_2-Induced Injury *In Vivo*

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Here we report our ongoing investigation of the cardiovascular effects of acetaminophen, with emphasis on oxidation-induced canine myocardial dysfunction. The objective of the current study was to investigate whether acetaminophen could attenuate exogenous H_2O_2-mediated myocardial dysfunction *in vivo*. Respiratory, metabolic, and hemodynamic indices such as left ventricular function (LVDP and $\pm dP/dt_{max}$), and percent ectopy were measured in anesthetized, open-chest dogs during intravenous administration of 0.88 mM, 2.2 mM, 6.6 mM H_2O_2. Following 6.6 mM H_2O_2, tissue from the left ventricle was harvested for electron microscopy. Left ventricular function did not vary significantly between vehicle and acetaminophen groups under baseline conditions. Acetaminophen-treated dogs regained a significantly greater fraction of baseline function after high concentrations of H_2O_2 than vehicle-treated dogs. Moreover, the incidence of H_2O_2-induced ventricular arrhythmias was significantly reduced in the acetaminophen-treated group. Percent ectopy following 6.6 mM concentrations of H_2O_2 was 1 ± 0.3 vs. 0.3 ± 0.1 ($P < 0.05$) for vehicle- and acetaminophen-treated dogs, respectively. Additionally, electron micrograph images of left ventricular tissue confirmed preservation of tissue ultrastructure in acetaminophen-treated hearts when compared to vehicle. We conclude that, in the canine myocardium, acetaminophen is both functionally cardioprotective and antiarrhythmic against H_2O_2-induced oxidative injury. Exp Biol Med 233:1315–1322, 2008

Key words: canine myocardium; ROS; oxidative stress; pathology; ischemia/reperfusion injury

Introduction

For over 100 years, acetaminophen has been exclusively used as an efficacious antipyretic and analgesic. Research surrounding this drug has concentrated primarily on these applications (1–3), but within the last few decades has begun to flourish. Spurred by the discovery of aspirin's beneficial effects on the cardiovascular system, investigators have begun using animal preparations to explore the unknown physiologic influences of acetaminophen on the mammalian cardiovascular system and its potential role in protection against oxidative injury.

A number of mechanisms have been shown to mediate post-ischemia, reperfusion-induced, myocardial injury. The most prevalent mechanism involves oxidative stress (4). During ischemia/reperfusion, reactive oxygen species (ROS) such as superoxide ($O_2\cdot$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH\cdot$), and peroxynitrite ($ONOO^-$) are synthesized and released exponentially. While low levels of oxidants are normal and necessary to carry out cellular functions (5), production and release of these agents are exponentially increased following ischemia/reperfusion and can trigger tissue injury and death (6–8). The proposed oxidant scavenging properties of acetaminophen interfere with many cellular cascades (i.e., apoptosis) which would otherwise be induced by injury-generated signaling of ROS (9).

Numerous publications have indirectly demonstrated acetaminophen's capacity to function as an antioxidant. In reperfused/reoxygenated isolated guinea pig hearts following ischemia/hypoxia, peroxynitrite-mediated chemiluminescence was attenuated in the presence of acetaminophen, concomitant with functional preservation (10, 11). More recently, Van Dyke and colleagues (12) describe the antioxidant capacity via reduction or complete abolishment of chemiluminescence in the L-012 and SIN-1 $ONOO^-$ generating system. Additionally, investigators have found other antioxidants effective in scavenging H_2O_2-mediated release of $OH\cdot$ during the Fenton reaction (13, 14).

Exogenous H_2O_2 is commonly used as a source of oxygen-derived free radicals, specifically $OH\cdot$, to study the

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effects of the reperfused ischemic myocardium (15, 16). In isolated guinea pig hearts, mechanical cardioprotection was reported during both H_2O_2-induced oxidative-injury and ischemia/reperfusion injury, in the presence of acetaminophen (10, 17–20). Similar attenuation by acetaminophen of exogenous $ONOO^-$-induced contractile dysfunction was observed in conjunction with decreased myocardial matrix metalloproteinase-2 (MMP-2) and troponin I production (21). Recent investigation of acetaminophen in our laboratory has provided similar evidence that acetaminophen is cardioprotective *in vivo*, exerting antiarrhythmic (22) and tissue ultrastructure (23) preservation during ischemia/reperfusion in dogs.

The objective of the current study was to determine whether acetaminophen could attenuate H_2O_2-mediated myocardial dysfunction in canine myocardium. The results indicate that acetaminophen is effective in preserving cardiac function and rhythm *in vivo* and protects specifically against this oxidant, which is a component of the oxidative mechanism for ischemia/reperfusion injury.

Materials and Methods

Animals. All experiments were performed on male mongrel dogs weighing 22 ± 0.5 kg. Dogs were obtained from licensed vendors, and housed in AAALAC-accredited facilities where room temperature, humidity, and lighting were controlled. Daily rations of Purina Dog Chow and water were provided *ad libitum*. Dogs were allowed several days to acclimate to their new housing conditions and were fasted for 24 h prior to the day of experimentation (water provided *ad libitum*). Institutional review and approval of protocols were obtained before initiating experiments and conform with the *Guide for the Care and Use of Laboratory Animals*.

Surgical Preparation and Instrumentation. Hair was clipped from the inguinal region, the chest, and on the fore- and hind-limbs in the vivarium, then dogs were transported to the experimental laboratory where they were anesthetized with propofol (6.5 mg/kg i.v.). Immediately following induction of general anesthesia, they were intubated and placed on isoflurane anesthesia (Aerrane, Isoflurane, USP, Baxter, Deerfield, IL) administered in supplemental oxygen at an MAC of 2.0 ± 0.5 per cent and flow rate of 2.0 ± 0.5 liters per minute (Matrix Calibrated Vaporizer, model VIP 3000, Orchard Park, NY; in-line with a Veterinary Anesthesia Ventilator, 2000 ml adult bellows, model 2002IE, Hallowell EMC, Pittsfield, MA).

The right femoral artery and vein were isolated and cannulated [polyethylene (PE)-240 catheters filled with 0.9% NaCl solution] so that monitoring of systemic arterial blood pressure (Pā, pulsatile and mean) and fluid administration (500 U/kg heparin, H_2O_2, acetaminophen and saline) could occur in each vessel respectively. A left-sided thoracotomy was performed and lobes of the left lung were retracted and the pericardium incised. A short, 18 g angiocatheter (BD Angiocath Autoguard, Sandy, UT) was then placed in the left ventricular chamber via the apical dimple. This was used to monitor left ventricular developed pressure (LVDP) and its derivative ($\pm dP/dt_{max}$). A standard 3-lead limb electrocardiogram was attached and used to determine heart rate. Dogs were then allowed time (~20 min) for monitored variables to achieve a steady state.

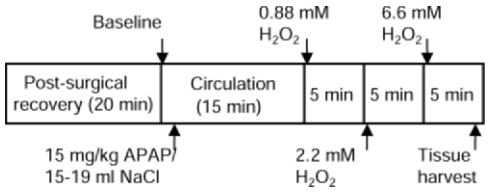

Figure 1. Protocol schematic. All dogs were allowed ~20 min to recover from surgery and achieve stable baseline status. Depending on the group, dogs were administered either 15 mg/kg acetaminophen or an equivalent volume of saline and allowed ~15 min to achieve a steady state. Both groups of dogs were administered a low (0.88 mM) concentration bolus of H_2O_2, followed 5 min later by a medium (2.2 mM) concentration bolus, followed 5 min later by a high (6.6 mM) concentration bolus of H_2O_2.

Experimental Protocol. Two groups of dogs were studied. Both groups were allowed ~20 min to recover from surgical invasion and achieve baseline status. One group was then pretreated with vehicle (0.9% NaCl, $n = 10$) and the other with acetaminophen (15 mg/kg, $n = 10$) (Fig. 1). Each pretreatment was administered as an intravenous bolus (15–19 ml), and allowed to circulate for ~15 min. Following the circulation period, both groups were administered incremental concentrations (0.88 mM, 2.2 mM, 6.6 mM) of H_2O_2 (0.88 M H_2O_2 solution, i.v., Target Corporation, Minneapolis, MN) at 5-min intervals via the femoral vein. All other variables were monitored continuously.

Determination of Plasma Concentration of Acetaminophen. In a third group of dogs ($n = 6$), one bolus injection of 15 mg/kg acetaminophen was given via the left femoral vein and allowed to circulate for 3 hours. Arterial blood samples (i.e., 2–3 ml each) were withdrawn at 0, 30, 60, 90, 120, 150, and 180 min after administration of acetaminophen. The blood samples were centrifuged at ~2,000 *g* for 10 min, and plasma stored at −70°C. The concentration of acetaminophen in the plasma was determined using validated fluorescence polarization immunoassay performed by a diagnostic laboratory.

Myofibrillar Ultrastructure. We examined the myofibrillar ultrastructure of the epi- and mid-myocardium using electron microscopy in vehicle- ($n = 2$) and acetaminophen-treated ($n = 2$) dogs. Hearts were arrested using 3 M KCl 5 minutes after administration of 6.6 mM H_2O_2. Immediately following the bolus, a transmural section of myocardium was extracted from the left ventricular free wall adjacent and distal to the third or fourth branch of the left anterior descending artery. Heart sections were then submerged in fixative, and blocks (2–3 mm^3) of epi- and mid-myocardium were cut. Tissue blocks were fixed with

Table 1. Metabolic and Hemodynamic Status of Anesthetized Dogs Treated with Hydrogen Peroxide (H_2O_2) in the Presence (A) or Absence (V) of Acetaminophen[a]

	Baseline		Baseline + APAP		0.88 mM H_2O_2		2.2 mM H_2O_2		6.6 mM H_2O_2	
	V	A	V	A	V	A	V	A	V	A
Tc (°C)	39	39	38	38	38	38	37 ± 1	38	37 ± 1	38
HR (cycles/min)	106±35	109±36	106±35	111±37	107±36	110±37	105±35	109±36	103±34	106±35
P_{O2} (mmHg)	381±26	386±31	381±26	386±31	388±25	376±28	373±28	366±29	320±33	316±31
P_{CO2} (mmHg)	41±1	45±1.6	41±1	45± 1.6	43±1.3	46±2.1	43±1.4	47±2.3	43±1.3	48±2.5
Na^+ (mEq/L)	135±9	141±1	135±9	141±1	143	142±1	143	142±1	142	141±1
K^+ (mEq/L)	4.2±0.1	4.1±0.1	4.2±0.1	4.1±0.1	4.2±0.1	4±0.1	4.1±0.1	4.1	4.1±0.1	4±0.1
Ca^{2+} (mEq/L)	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4

[a] Data are mean ± SEM ($n = 10$). V, vehicle; A, acetaminophen; Pā, systemic mean arterial blood pressure; HR, heart rate; PO_2, partial pressure of oxygen; PCO_2, partial pressure of carbon dioxide; Na^+, sodium; K^+, potassium; Ca^{2+}, calcium.

Trumps fixative for 2 hours then 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 Ltd, Japan) using standard methods. Electronmicroscopic images were digitally acquired and examined for signs of tissue damage.

Monitored Variables and Data Acquisition. For each protocol group, monitored indices included: core temperature (C_t, °C), ventilatory frequency (Vf, cycles/min), tidal volume (Vt, ml), end-tidal CO_2 (percent of expired gases, model NPB-75, Nellcor Puritan Bennett capnograph, Pleasanton, CA), oxyhemoglobin saturation (SaO_2, %, capnography, model NPB-75), blood gasses (PO_2, PCO_2, mmHg, model 248 blood gases/pH analyzer, Chiron Diagnostics, West Haven, CT), electrolytes (K^+, Ca^{2+}, Na^+, EasyLyte Calcium, Medica, Bedford, MA), systemic mean arterial pressure (Pā, mmHg), LVDP (mmHg), and its $\pm dP/dt_{max}$ (mmHg/s), heart rate (HR, cycles/min), and the ECG. All 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).

Statistics. All data are presented as mean ± standard error of the mean (SEM). The experiments were designed *a priori*, and Student's *t* test for nonpaired replicates was used to identify statistically significant differences between treatment means. Between-group variability was compared using one-way ANOVA followed by Tukey's test for means comparison. Significance was accepted at $P < 0.05$.

Results

Hemodynamic and Mechanical Properties. Hemodynamic, respiratory, and metabolic indices such as heart rate (HR), partial pressure of oxygen (P_{O2}), and partial pressure of carbon dioxide (P_{CO2}) did not vary significantly between vehicle- and acetaminophen-treated dogs under baseline or experimental conditions. Circulating Na^+, K^+, and Ca^{2+} levels were also constant throughout the experiment (Table 1). Reported hemodynamic and functional data were analyzed during the first 30 sec after administration of H_2O_2, the time-point that resulted in the most significant cardiovascular responses (Fig. 2). Arterial blood samples were collected 5 min after administration of H_2O_2 to allow time for complete mixing and circulation.

At all concentrations of H_2O_2, acetaminophen-treated dogs showed significant preservation of left ventricular function (Figs. 3–4). Left ventricular developed pressure (LVDP) and its derivative ($\pm dP/dt_{max}$) at baseline did not vary significantly between vehicle- and acetaminophen-treated dogs. A significant decrease in LVDP was seen in vehicle-treated dogs after treatment with 6.6 mM H_2O_2 as compared with baseline. This LVDP decrease from baseline

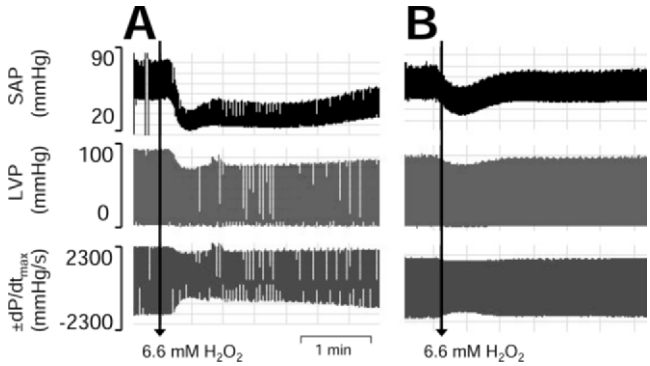

Figure 2. Representative trace of systemic arterial pressure (SAP), left ventricular pressure (LVP) and its derivative ($\pm dP/dt_{max}$) in vehicle- (A) and acetaminophen-treated (B) dogs following administration of 6.6 mM H_2O_2.

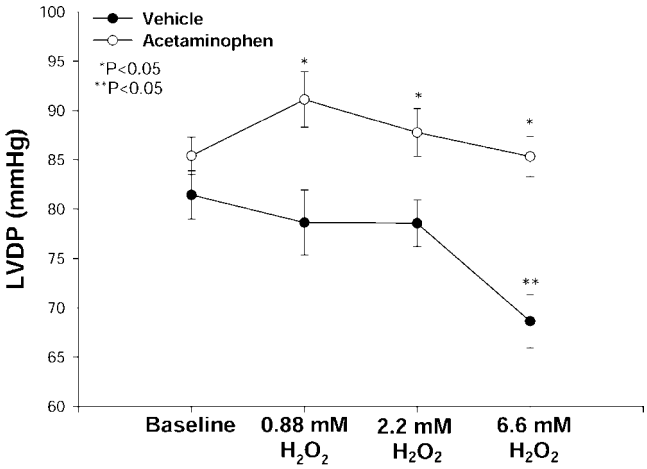

Figure 3. LVDP was retained in acetaminophen- [15 mg/kg] versus vehicle-treated dogs ($n = 10$ per group) during administration of increasing concentrations of H_2O_2. * $P < 0.05$ relative to corresponding value for vehicle treatment. ** $P < 0.05$ relative to baseline value for vehicle treatment.

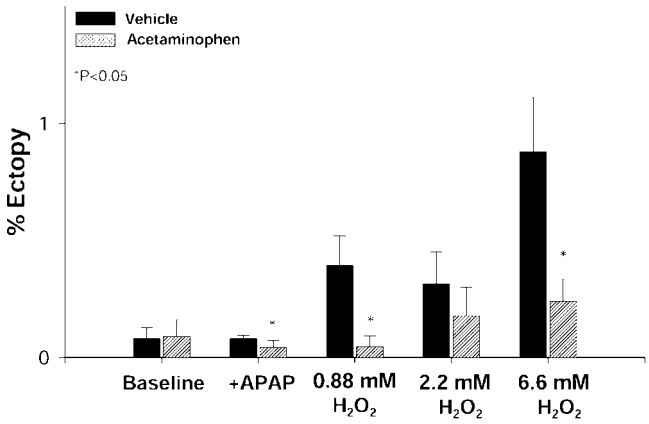

Figure 5. Influence of acetaminophen- [15 mg/kg] or vehicle-treatment on H_2O_2-induced arrhythmias ($n = 10$ per group). After 0.88 mM and 6.6 mM of hydrogen peroxide, acetaminophen-treated groups significantly retained a more regular rhythm than vehicle-treated groups. * $P < 0.05$ relative to corresponding value for vehicle treatment.

was not present in acetaminophen-treated dogs after treatment with 6.6 mM H_2O_2. Specifically, after treatment with 6.6 mM H_2O_2, pressures were 68 ± 3 for vehicle and 79 ± 4 mmHg ($P < 0.05$) for acetaminophen-treated dogs (Fig. 3).

Systolic ($+dP/dt_{max}$) and diastolic ($-dP/dt_{max}$) function were also preserved in acetaminophen-versus vehicle-treated hearts. Whereas $+dP/dt_{max}$ of vehicle-treated animals dropped from 1545 ± 169 to 1355 ± 141 mmHg/s following administration of 6.6 mM H_2O_2, acetaminophen-treated animals showed a significant increase, from 1711 ± 90 to 1846 ± 144 mmHg/s (Fig. 4).

Electrocardiogram. The severity of arrhythmias was expressed as the percent ectopy: the number of ectopic beats (per min) divided by the total beats (per min) × 100

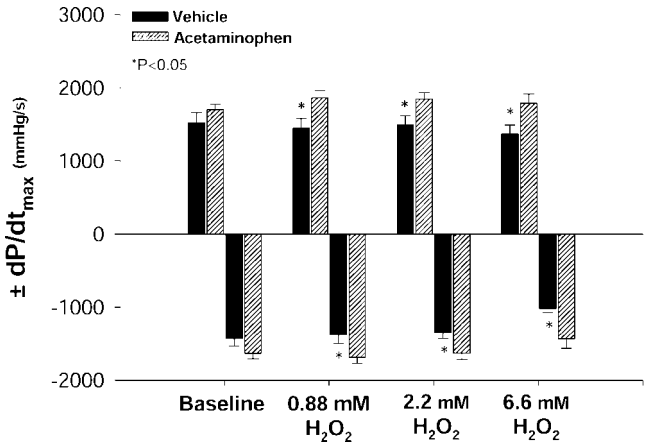

Figure 4. Influence of administration of H_2O_2 on $\pm dP/dt_{max}$ in acetaminophen- [15 mg/kg] or vehicle-treated dogs ($n = 10$ per group). Acetaminophen treated groups gained or retained systolic function and retained diastolic function compared to vehicle treated groups. * $P < 0.05$ relative to corresponding value for vehicle treatment.

(24). The ectopic beats were evaluated from the shape of the QRS complex and its associated relationship to systemic and ventricular pressures based on previously defined guidelines (25).

Heart rate and incidence of ventricular arrhythmias did not vary between groups under baseline conditions. A H_2O_2 concentration-dependent increase in arrhythmias was seen in both vehicle- and acetaminophen-treated dogs, but was significantly attenuated in the presence of acetaminophen. For example, after intravenous administration of 0.88 mM H_2O_2, percent ectopy was 0.39 ± 0.13 in vehicle- and 0.05 ± 0.02 in acetaminophen-treated dogs. Following treatment with 6.6 mM, percent ectopy was 1.04 ± 0.3 in vehicle- and 0.27 ± 0.1 ($P < 0.05$) in acetaminophen-treated dogs (Fig. 5). This is a 77% decrease in total number of ectopic beats in acetaminophen-treated dogs when compared to vehicle-treated dogs.

Additionally, the occurrence of basal arrhythmia (reflecting the recovery from the surgical intervention) did not vary significantly between vehicle- and acetaminophen-treated groups. However, immediately following administration of 15 mg/kg acetaminophen, percent ectopy decreased significantly in the acetaminophen-treated dogs in the absence of H_2O_2 (Fig. 5).

Plasma Concentration of Acetaminophen. The time course for changes in the circulating concentrations of acetaminophen after i.v. administration is shown in Figure 6. The elimination half-life ($t_{1/2}$) of a 15 mg/kg acetaminophen bolus was an estimated 55 min. The maximum plasma concentration (Cmax) and the time corresponding to Cmax (Tmax) were 19.1 ± 2 ug/ml and 30 min.

Electron Microscopy. Following 6.6 mM treatment of H_2O_2, epi-myocardial tissue obtained from vehicle-treated hearts showed dilated sarcotubules, mild contracture, liposome deposition, and numerous mitochondrial amor-

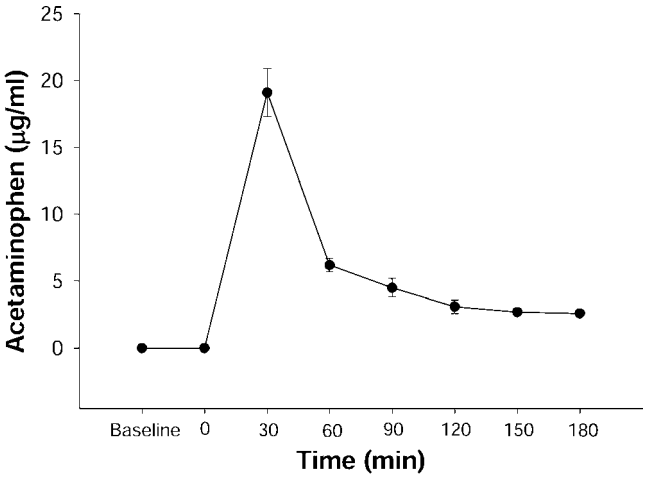

Figure 6. Plasma concentrations of circulating acetaminophen after i.v. administration [15 mg/kg]. Peak concentration (19.1 ± 2 ug/ml) was found 30 min after administration, and nearly completely metabolized after 3 hours.

phous matrix densities (AMDs) (Fig. 7B). The light I-bands and dark Z-lines within the sarcomere were disjointed and blurred in vehicle-treated hearts but well defined in acetaminophen-treated hearts. Acetaminophen-treated hearts had less lipid deposition, an absence of vacuoles, more compact cristae and more ordered myofilaments (Fig. 7A). Also, the mitochondria did not appear as swollen, were darker, lacked AMDs, and were more densely packed. Examination of mid-myocardial tissue exhibited similar signs of damage and protection in vehicle- (Fig. 7D) and acetaminophen-treated (Fig. 7C) dogs respectively.

Discussion

In this study, we examined the structural, functional, and electrical characteristics of the canine heart following H_2O_2-mediated oxidative damage, and the potential of acetaminophen to impact these variables.

One mechanism of cardiovascular damage following ischemia/reperfusion is the overabundance of ROS, which damage cardiomyocyte lipid membranes, nucleic acids, protein, carbohydrates, and interfere with intra- and inter-

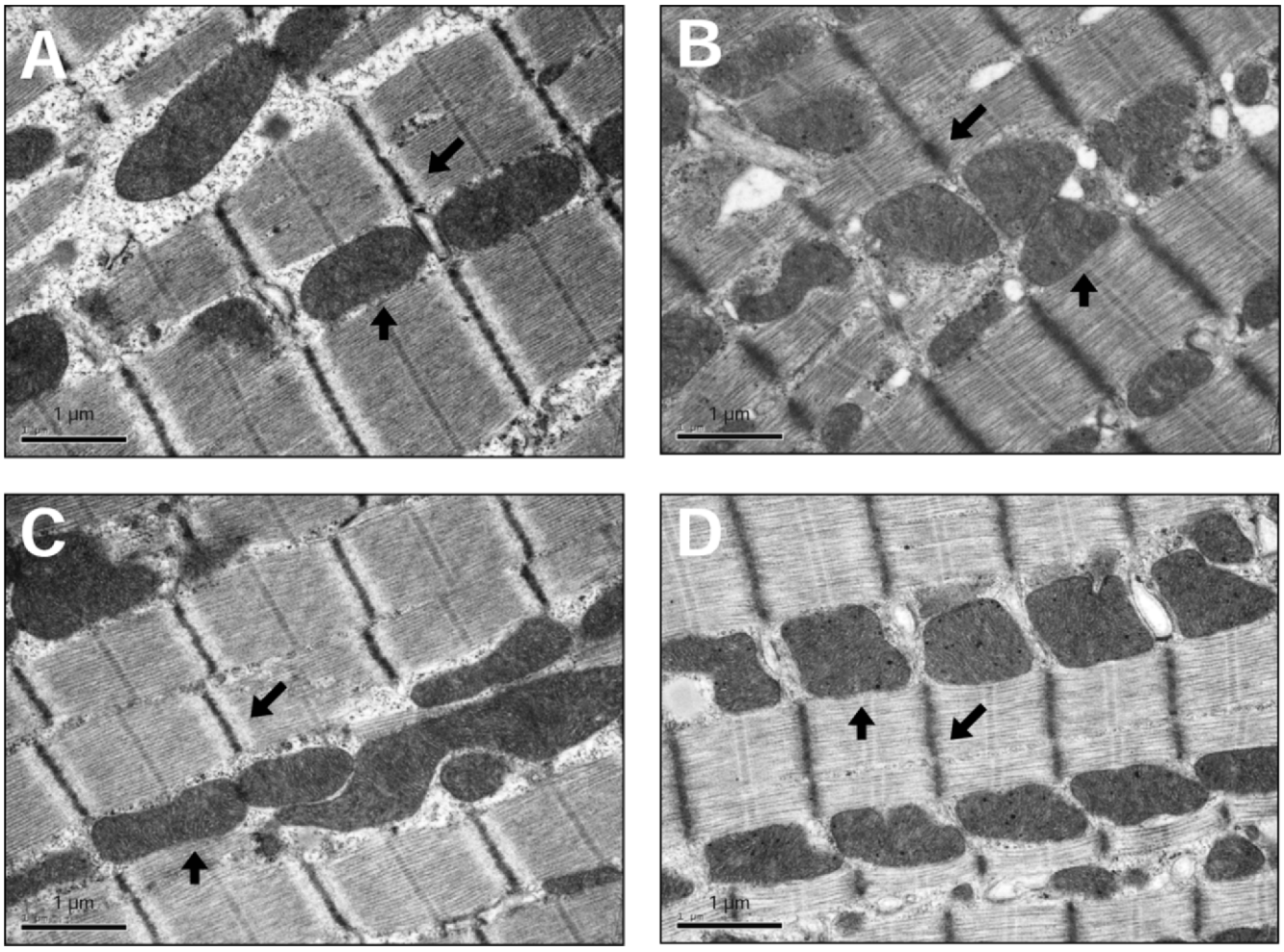

Figure 7. Electron micrographs (×14,000) of canine left ventricular free wall epi- (A and B) and mid-myocardium (C and D) after treatment with 6.6 mM H_2O_2 in acetaminophen- (A and C) and vehicle-treated (B and D) dogs. Note the mitochondria and light/dark bands of sarcomeres.

cellular signaling pathways (26, 27). The most prevalent forms of ROS in the myocardium are $O_2\cdot$, H_2O_2, $OH\cdot$, and $ONOO^-$. Under physiologic conditions, ROS are produced in moderation during cellular respiration allowing endogenous antioxidant defenses (i.e., catalase, SOD, and glutathione) to neutralize these compounds before causing irreversible damage.

Physiologic ([μM]) levels of H_2O_2 can either act as ubiquitous second messengers, or directly target intracellular components such as ion channels/transporters and contractile proteins (28), and be decomposed by catalase and other peroxidases into molecular oxygen and water. At high μM to low mM concentrations, catalase and peroxidases become saturated allowing H_2O_2 to react readily with iron-linked substances, producing even stronger oxidizers, $OH\cdot$ and peroxide (29). These compounds, in addition to H_2O_2, are responsible for oxidant-induced calcium overload (14, 16) mediated by a variety of mechanisms. *In vitro*, H_2O_2 has been demonstrated to activate Na^+-Ca^{2+} exchange, either fatigue (30) or enhance L-type Ca^{2+} currents (16), increase Na^+-H^+ exchange (31, 32), and induce Ca^{2+}-induced Ca^{2+} release from the SR (33) in cardiac myocytes. The present study aimed to identify the effects of acetaminophen on H_2O_2-induced cardiomyocyte injury *in vivo*.

We have previously reported that acetaminophen reduced infarct size in the canine myocardium following regional ischemia/reperfusion, and that this was accomplished concurrent with a significant reduction in peroxynitrite (23). Additionally, Merrill *et al*. (22) recently reported acetaminophen-mediated attenuation of both ischemia/reperfusion- and ouabain-induced ventricular arrhythmia in the dog.

Experimental Protocols. Experiments were conducted to find concentrations of H_2O_2 that would elicit inotropic effects but that would not arrest the heart (data not shown). In most cases we observed a negligible, negative inotropic effect with a concentration of 0.88 mM or smaller, variable inotropic responses at 2.2 mM, and significant changes at concentrations of 6.6 mM, 8.8 mM, and 13.2 mM. Thus we settled on a low (0.88 mM), mid (2.2 mM), and high (6.6 mM) dose to account for inter-dog sensitivity and variability.

Hemodynamic and Mechanical Properties. A mild dose-dependent biphasic effect was observed in contractile force following treatment with H_2O_2 (data not shown). At μM concentrations there was an initial positive inotropic effect, followed by negative inotropy at mM concentrations. However, after treatment with all concentrations of H_2O_2, acetaminophen-treated dogs showed greater preservation of baseline function when compared to vehicle. Systolic function increased within 10 minutes of acetaminophen administration, and remained elevated above baseline levels during H_2O_2 treatment. This unanticipated increase in contractility following treatment with acetaminophen is indicative of positive inotropic compounds, in particular, cardiac glycosides which inhibit the Na^+/K^+ pump and ultimately increase myocyte contractility (34).

Diastolic function did not significantly decrease in acetaminophen-treated dogs following treatment with H_2O_2. However, in vehicle-treated dogs following 6.6 mM H_2O_2, diastolic function significantly declined to 68% of baseline. This decreased function is indicative of diminished sarcomere relaxation, a common effect of Ca^{2+} overload, which is absent in acetaminophen-treated dogs. Similar acetaminophen-mediated diastolic preservation was recently observed during hypoxia/reoxygenation in a Langendorf preparation of guinea pig hearts (21). However, since we did not consider peripheral vascular resistance in these sets of experiments, we cannot rule out the possible effects of acetaminophen on cardiac output, resistance or any other variables relating directly or indirectly to them. To our knowledge, there are no reports of vasoconstriction or hypertension linked to acetaminophen consumption, which would reflect the change in $-dP/dt_{max}$.

Attenuation of H_2O_2-induced lipid peroxidation of myocyte membrane ion channels by acetaminophen, could be partially responsible for the results of the current study. Oxidation-induced lipid peroxidation of myocytes is responsible for a wide variety of pathology following myocardial infarction. Lipid peroxidation by ROS often results in damage to Na^+/K^+ pumps (35, 36), Na^+-H^+ exchangers (37–39), and Na^+ (40) and Ca^+ (41) channels, as well as other membrane bound proteins (40). Recently, Davidenko (42) reported that the reaction of peroxidase in the presence of a phenol leads to polymerization of the phenol, increasing antioxidant capacity, and inactivation of the oxidant. This and other antioxidant studies (20) corroborate our hypothesis that because acetaminophen is a mono-phenol, it can scavenge ROS and protect channels in part by preventing lipid peroxidation.

Electrocardiogram. Overall, a significant antiarrhythmic effect was observed in acetaminophen- versus vehicle-treated dogs following treatment with H_2O_2. Using exogenous ouabain to induce arrhythmia in dogs, we recently reported similar antiarrhythmic activity of acetaminophen (22), suggesting that acetaminophen's mechanism of protection may involve the Na^+/K^+ pump. Other groups have reported a decrease in Na^+/K^+ pump activity following H_2O_2, which is effectively abolished following treatment with other antioxidants (43). Since mammalian myocardium contains peroxidase and catalase which react with H_2O_2 to produce strong oxidants, it is not surprising that H_2O_2 causes cardiac damage and that the antioxidant phenolic properties of acetaminophen minimize the damage to both myocytes and mitochondria. Based on these studies, we hypothesize that acetaminophen attenuates arrhythmias, at least in part, by protecting ion channels and transporters, e.g. the Na^+/K^+ pump, from oxidant damage. More work is needed to clarify the specific

target(s) of acetaminophen and its contribution to anti-arrhythmogenesis.

Plasma Concentration of Acetaminophen. We chose a dose of 15 mg/kg acetaminophen as a therapeutic concentration in the dog based on the maximum recommended daily dose of Extra Strength Tylenol for humans, 60 mg/kg (20, 44), and on previous clinical studies (1). Previous studies completed in this laboratory have routinely utilized a divided dose, 15 mg/kg before ischemia and another 15 mg/kg mid-way through reperfusion for a total of 30 mg/kg (23). The suggested human therapeutic range is 650–1000 mg orally or 10–100 μg/ml in plasma depending on the study design, route of administration, etc. (44–46). Thus, our concentration is in the lower therapeutic range, and is almost completely metabolized in the dog after 3 hours with a peak concentration of 19.1 ± 2 ug/ml at 30 min post administration.

Myofibrillar Ultrastructure. Scanning electron micrographs (SEM) of the epi- and mid-myocardium showed a trend in tissue myofibrillar ultrastructure and mitochondrial preservation in acetaminophen-treated dogs when compared to vehicle. Vehicle-treated hearts showed dilated sarcotubules and numerous vacuoles, which were absent in acetaminophen-treated dogs. Contraction bands and liposomes were ubiquitous in vehicle-treated hearts, and nearly absent in acetaminophen-treated dogs. The light I-bands and dark Z-lines within the sarcomere were disjointed and blurred in vehicle-treated hearts but well defined in acetaminophen-treated hearts. Mitochondria were swollen, rounded, diffusely packed, and contained AMDs in the presence of vehicle. With acetaminophen treatment, mitochondria were elongated, non-swollen, dark, lacked AMDs, and were more densely packed. These defining features of damage are indicative of compromised myocyte membrane structure, function and increased mitochondrial permeability, consistent with a higher degree of cellular damage in vehicle-treated hearts versus acetaminophen-treated (9, 47, 48).

Concentration of H_2O_2. Previous studies in smaller mammals and cell culture have utilized H_2O_2 concentrations equivalent to the ones we chose. Common concentrations range from 100 μM to 1 mM (10, 14, 49, 50) depending on the experimental model. The effects of H_2O_2 have also been tested in the diseased human heart. Loh and colleagues (51) tested H_2O_2 on the heart and found a negligible change in cardiac function using 1 mM H_2O_2 and a pronounced effect with a 3 mM solution. For this reason, we chose a range of concentrations to elicit variable effects in the intact canine heart.

Direct measurement of H_2O_2 or OH· in the blood was not performed in these experiments; we estimated the concentration of circulating H_2O_2 in the blood based on the animals' weight. We estimated that after 0.88 mM of H_2O_2 the concentration of H_2O_2 in the dog was 0.5 mM, after 2.2 mM it was 1.2 mM, and after 6.6 mM the circulating concentration was 3.78 mM. Of course, these estimates do not account for either the kinetics or volume of distribution of H_2O_2 in body water compartments.

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