Chronically Administered Acetaminophen and the Ischemia/Reperfused Myocardium

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Male and female Hartley strain guinea pigs weighing 280 ± 10 g were given acetaminophen-treated water *ad libitum* for 10 days. Sham-treated control animals were given similar quantities of untreated tap water (vehicle-treated control group). On Day 10, hearts were extracted, instrumented, and exposed to an ischemia (low-flow, 20 min)/reperfusion protocol. Our objective was to compare and contrast ventricular function, coronary circulation, and selected biochemical and histological indices in the two treatment groups. Left ventricular developed pressure in the early minutes of reperfusion was significantly greater in the presence of acetaminophen, e.g., at 1 min, 40 ± 4 vs 21 ± 3 mmHg ($P < 0.05$). Coronary perfusion pressure was significantly less from 3 to 40 min of reperfusion in the presence of acetaminophen. Creatine kinase release in vehicle-treated hearts rose from 42 ± 14 (baseline) to 78 ± 25 units/liter by the end of ischemia. Corresponding values in acetaminophen-treated hearts were 36 ± 8 and 44 ± 14 units/liter. Acetaminophen significantly ($P < 0.05$) attenuated release of creatine kinase. Chemiluminescence, an indicator of the *in vitro* production of peroxynitrite via the *in vivo* release of superoxide and nitric oxide, was also significantly attenuated by acetaminophen. Electron microscopy indicated a well-preserved myofibrillar ultrastructure in the postischemic myocardium of acetaminophen-treated hearts relative to vehicle-treated hearts (e.g., few signs of contraction bands, little or no evidence of swollen mitochondria, and well-defined light and dark bands in sarcomeres with acetaminophen; opposite with vehicle). We conclude that chronic administration of acetaminophen provides cardioprotection to the postischemic, reperfused rodent myocardium. Exp Biol Med 228:674–682, 2003

Key words: myocardial ultrastructure; cardioprotection; creatine kinase; peroxynitrite

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Acetaminophen has been used in mainstream Western medicine for many decades (1, 2), but thorough, physiological investigations of its actions/mechanisms of action at the mammalian, organ systems level have not been undertaken. We began investigating the cardiovascular properties of acetaminophen a few years ago (3–5), and have focused on its cardioprotective efficacy and antioxidant mechanisms in the ischemia/reperfused, rodent myocardium.

Acetaminophen has a tyrosine-like structure (phenolic)) similar to that of vitamin E (a-tocopherol) (6–11) and might behave like a scavenger/neutralizer of damaging oxidants. Acutely administered acetaminophen has been reported recently to inhibit myeloperoxidase oxidation of LDL (12), and we have indirect evidence that it inhibits myocardial release of hydroxyl radicals and precursors of peroxynitrite in the early minutes of reperfusion (4, 5). Acute administration of acetaminophen can attenuate the dose-dependent negative inotropic effects of hydrogen peroxide in the Langendorff-perfused rodent heart (5), but it might also act in a pro-oxidant fashion by generating phenoxyl radicals (10).

The actions of chronically administered acetaminophen on the mammalian myocardium have not been studied. The main purpose of the current investigation was to administer acetaminophen chronically, and then to study its potential for cardioprotection in the postischemic reperfused mammalian myocardium. Results of such an investigation, coupled with those obtained during its acute administration, will give a more complete picture of the previously unknown cardiovascular actions of this important therapeutic agent.

Materials and Methods

Animals. We used the isolated guinea pig heart preparation described three decades ago by Bunger *et al.* (13–15).

Briefly, Hartley strain guinea pigs, in equal numbers of males and females, weighing about 280 ± 10 g were used. They were housed individually in a room that was regulated for temperature, humidity, and light:dark cycle (12:12-hr). Laboratory Animal Services of Rutgers University took care

of the daily maintenance of the guinea pigs. Study protocols were previously reviewed and approved by the Rutgers University IACUC. The authors subscribe to the Guide for the Care and Use of Laboratory Animals.

Chronic Administration of Acetaminophen. Acetaminophen in a final concentration of 0.35 mmol/l was added to the drinking water of the guinea pigs, and they were allowed to consume water *ad libitum* (plain tap water was provided *ad libitum* to vehicle-treated, control animals). The concentration of 0.35 m*M* was chosen because of its use in our prior studies (3–5). The acetaminophen-treated drinking water was colorless, odorless, and tasteless (personal experience, G.F. Merrill). Animals were housed individually and daily consumption of food and water was monitored. After 10 days, animals were brought to the laboratory and were investigated as described below. The period of 10 days was chosen because it is more than adequate for physiologic body water to be replaced with acetaminophen-containing water. Because acetaminophen is both water and lipid soluble (1, 2), we assumed it would have access to all three body water compartments (i.e., including the intracellular space).

Isolation and Instrumentation of the Guinea Pig Heart. After 10 days of treatment, the guinea pigs were euthanized by cranial crushing as approved by the AVMA Panel on Euthanasia and the Rutgers University IACUC. The main feature of the Langendorff preparation described by Bunger *et al.* (13, 14) and used in our laboratory is the *in situ* methodology (for a thorough discussion, see Ref. 16). After hearts were extracted and attached to the perfusion apparatus, coronary perfusate flow (retrograde aortic flow) was restored incrementally to 7 ml/min via an inline roller pump (model Peri Star 291; World Precision Instruments, Sarasota, FL). Coronary perfusion pressure was continuously monitored approximately 1-2 cm upstream to the cannulated aorta (23G needle, PE90 cannula, P231D pressure transducer; Gould-Statham, Oxnard, CA). Left ventricular pressure and $\pm dP/dt_{max}$ (indices of contractility) were monitored by passing a flaccid, latex rubber balloon through the left atrium, across the mitral valve, and into the chamber of the left ventricle. The balloon was filled to a final volume of 75 to 125 μl using Krebs-Henseleit physiologic salt solution (KHB; corresponding to a late diastolic pressure of 0-5 mmHg). Pacing/recording electrodes were attached to the base of the ventricles, and hearts were paced at a rate equal to the spontaneous rate plus 15%.

KHB Solution. Perfusate was a modified KHB of the following composition (in millimoles): glucose 10.0, pyruvate 2.0, NaCl 127.5, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.5, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 24.9, and insulin 10.0 mU/ml. The KHB was equilibrated with 95% O_2 and 5% CO_2 to yield a final pH of approximately 7.40 ± 0.02 and was warmed to 38°C (water-jacketed perfusion system; model 1112 hearter/circulator; Polyscience, Niles, IL). Perfusate gases (PO_2 and PCO_2) and pH were monitored regularly by collecting samples anaerobically and immediately analyzing them (blood gases/pH analyzer, model 248; Bayer Diagnostics, Norwood, MA).

Monitored variables included: heart rate (HR, cycles/min, cpm), coronary perfusate flow rate (CPF, ml/min; this was retrograde aortic flow and was assumed to equal antegrade coronary flow; no recirculation), coronary perfusion pressure (CPP, mmHg), coronary vascular resistance (CVR, mmHg/ml/min, calculated), indices of left ventricular mechanical function including, isovolumetric left ventricular peak systolic pressure (LVPs, mmHg), left ventricular late diastolic pressure (LVPd, mmHg), left ventricular developed pressure (LVDP, mmHg; the difference between LVPs and LVPd), pressure rate product (LVDP × HR, mmHg/min), and the first derivative of left ventricular pressure ($+dP/dt_{max}$, mmHg/sec).

Experimental Protocol. Hearts were divided into two groups, acetaminophen-treated and vehicle-treated. After hearts were extracted and instrumented, they were allowed 30 min for monitored variables to reach their steady states. Subsequently, baseline control data were collected and a 20-min period of low-flow, global myocardial ischemia was initiated. Ischemia was achieved by reducing the pump flow rate from 7.0 to 1.0 ml/min. Maintaining some flow during ischemia enabled us to collect venous effluent samples for chemical/blood gases/pH analysis. Recorded variables were monitored continuously but are reported here for only 10 and 20 min of ischemia. After the 20 min of ischemia, the pump rate was restored to 7.0 ml/min and reperfusion was initiated. Reperfusion data were monitored continuously but are reported here only for 1, 3, 6, 10, and 40 min. These times were selected because they are consistent with our previously reported results (3–5), they enabled us to collect early (e.g., 1 and 3 min) and late (e.g., 40 min) reperfusion data, and they cover a reasonable fraction of the period of impaired mechanical function for this particular experimental design.

Physiologically, we were most interested in comparing and contrasting indices of both mechanical and circulatory function. For these, we focused on CPP and CVR (because we controlled CPF), and on $\pm dP/dt_{max}$ as an indicator of ventricular contractility. A sample of $n = 10$ hearts was used in each treatment group.

Creatine Kinase. Creatine kinase is an accepted biochemical marker of myocardial injury during ischemia and reperfusion (17–19). In this experiment, we compared the effects of chronic administration of both acetaminophen and vehicle on the myocardial release of creatine kinase ($n = 8$ hearts per group). Standard assays (product no. 47-10; Sigma Diagnostics, St. Louis, MO) were used and were based on the modified procedures of Nielsen (20) and Rosalki (21). Briefly, coronary venous effluent samples of perfusate (0.5-1.0 ml each) were collected under baseline control conditions, at 10 and 20 min of ischemia, and at 1, 3, 6, 10, and 40 min of reperfusion. One milliliter of reconstituted reagent was added to 20 μl of venous effluent sample in a 1.0-ml minimum cuvette. Each sample was incubated

for 3 min at 30°C and was processed spectrophotometrically at 340 nm at 30-sec intervals for 120 sec. Data are expressed in units per liter, where one unit of activity is defined as the amount of enzyme that produces 1.0 μmol/min of NADH.

Peroxynitrite and Chemiluminescence. Ten additional hearts were used to evaluate the effects of chronically administered acetaminophen on the chemiluminescent potential of coronary venous effluent samples as previously reported (5). Hearts were subdivided into two treatment groups (n = 5 each), vehicle- and acetaminophen-treated (0.35 m*M*). Briefly, SIN-1 and luminol were added to each of the venous effluent samples before they were placed in the luminometer (model LB9505C; Berthold). SIN-1 generates peroxynitrite via superoxide and nitric oxide. In turn, peroxynitrite oxidizes luminol, producing a burst of blue light that is captured and quantified by computer.

Electron Microscopy. A separate group of chronically treated hearts (n = 6 vehicle-treated; n = 6 acetaminophen-treated) was used to assess myofibrillar ultrastructure. These hearts were exposed to the same experimental conditions as described above except that they were perfused with Karnovsky's fixative for 1 min under baseline control conditions (i.e., once recorded variables had achieved the steady state after 30 min perfusion with KHB, n = 2 per treatment group), after 20 min of ischemia (n = 2 per treatment group), and after ischemia plus 40 min of reperfusion (n = 2 per treatment group). Hearts were then submerged in fixative and blocks (1-2 mm^3) of myocardium were removed from the anterior free wall of the left ventricle midway between the left anterior descending and left ventricular branches of the left main coronary artery, about equidistance between base and apex. Blocks 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 (22).

Statistical Analysis. Two groups of hearts were studied: those taken from vehicle-treated, control animals (vehicle group), and those taken from acetaminophen-treated animals (experimental group). Within each group, results were initially analyzed using analysis of variance (ANOVA) for repeated measures to identify variability. Subsequently, an *a priori* test (Tukey's w procedure) was used to compare means between the two treatment groups.

Statistically significant differences between means were established at $P < 0.05$. All data are reported as means ± 1 SEM.

Results

Water Consumption. There were no statistically significant differences in the amounts of water consumed by animals in the two treatment groups. The acetaminophen-treated water was colorless, odorless, and tasteless (personal experience, G.F. Merrill). The presence of acetaminophen in the drinking water did not affect water consumption. During the 10-day treatment period, vehicle- and acetaminophen-treated animals consumed 81 ± 7 and 79 ± 5 ml of water per day, respectively (i.e., approximately 25-30 ml/100 g body weight/day). Corresponding changes in body weight during the 10-day period were 71 ± 4 and 70 ± 6 g. These numbers are consistent with Charles River guinea pig growth data. There were no statistically significant differences in the two groups. Neither did they display any behavioral characteristics that could be construed as an adverse effect of water consumption.

Left Ventricular Mechanical Function. There were no differences in left ventricular mechanical function between the two groups of hearts under baseline control conditions or during ischemia. During the 40 min of reperfusion, $+dP/dt_{max}$ of acetaminophen-treated hearts was significantly greater ($P < 0.05$) than that of vehicle-treated hearts at each time interval (Fig. 1). At baseline, $+dP/dt_{max}$ was 570 ± 75 and 591 ± 82 mmHg/sec in acetaminophen- and vehicle-treated hearts, respectively. At 1 min of reperfusion, corresponding values were 622 ± 68 vs 436 ± 84 mmHg/sec ($P < 0.05$). Not only is the value at 1 min of reperfusion for acetaminophen significantly greater than that in vehicle-treated hearts, it is noticeably elevated above its own baseline control value ($P < 0.05$). This trend between the two groups was maintained during the period of reperfusion. Similar results were observed for other indices of left ventricular mechanical function and are summarized in Table I.

Coronary Circulatory Function. Under baseline control conditions, there were no differences in CPF, CPP, or calculated CVR in the two groups (Table I). By 20 min of ischemia, CPP in vehicle- and acetaminophen-treated hearts showed no statistically significant differences. During the 40 min of reperfusion, the overall trend was toward significant coronary vasoconstriction in the presence of vehicle (Fig. 2). No such trend was observed in acetaminophen-treated hearts. At most time intervals during reperfusion, values for vehicle were significantly greater than corresponding values for acetaminophen, and also significantly greater than their own baseline control values.

Perfusate Gases and pH. Arterial perfusate gases under baseline control conditions (vehicle-treated hearts) displayed values in the typical ranges as follows: pH (7.42 ± 0.04 units), PCO_2 (36 ± 4 mmHg), and PO_2 (578 ± 26 mmHg). There were no significant differences between values in vehicle- and acetaminophen-treated hearts. Corresponding values for coronary venous effluent samples were: pH (7.32 ± 0.02 units), PCO_2 (44 ± 2 mmHg), and PO_2 (268 ± 54 mmHg). Again, there were no differences between groups. During ischemia, pH and PO_2 in the venous effluent decreased significantly to similar values in both groups, whereas PCO_2 increased correspondingly. By 40 min of reperfusion, venous effluent pH and PO_2 were modestly but significantly higher in the presence of acetaminophen, whereas PCO_2 was lower.

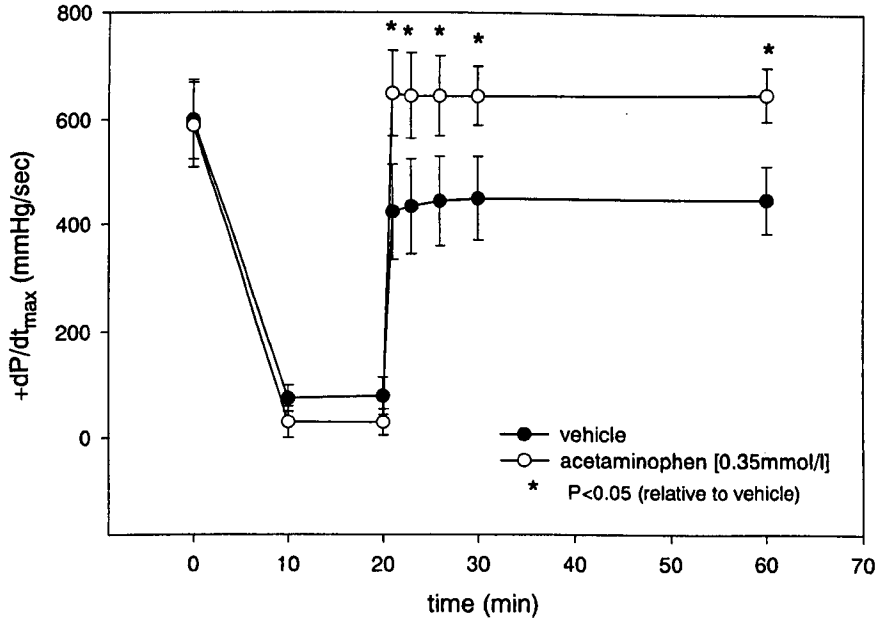

Figure 1. $+dP/dt_{max}$ (an index of ventricular contractility) is only one of several variables used to characterize left ventricular mechanical function. Baseline (control) data were collected at 0 min. Data during ischemia were collected at 10 and 20 min, and during reperfusion at 1, 3, 6, 10, and 40 min.

Table I. Influence of Ischemia/Reperfusion, in the Presence of Chronically Administered Acetaminophen, on Selected Cardiovascular Variables in the Perfused Guinea Pig Heart

	Ischemia			Reperfusion				
	Baseline	I10	I20	R1	R3	R6	R10	R40
Vehicle treated								
HR	229 ± 8	229 ± 8	229 ± 8	229 ± 8	229 ± 8	229 ± 8	229 ± 8	229 ± 8
CPP	46 ± 4	10 ± 2	8 ± 3	38 ± 4	48 ± 6	56 ± 8	64 ± 4#	74 ± 12#
CVR	7 ± 1	10 ± 1	8 ± 3	5 ± 2	7 ± 2	8 ± 2	9 ± 2	11 ± 2#
PRP	8000 ± 480	228 ± 23	153 ± 15	4740 ± 1221	4452 ± 1012	5072 ± 963	5600 ± 1045	6850 ± 1020
LVDP	36 ± 2	2 ± 1	1 ± 1	21 ± 3#	19 ± 3#	22 ± 2#	24 ± 2#	28 ± 3#
Acetaminophen treated								
HR	228 ± 3	228 ± 3	228 ± 3	228 ± 3	228 ± 3	228 ± 3	228 ± 3	228 ± 3
CPP	48 ± 4	8 ± 2	8 ± 3	32 ± 2#	36 ± 2*	42 ± 3*	46 ± 4*	50 ± 2*
CVR	7 ± 1	8 ± 1	8 ± 1	4 ± 1	5 ± 1*	6 ± 1*	7 ± 1*	7 ± 1*
PRP	8050 ± 540	260 ± 25	242 ± 32	9155 ± 976*	8910 ± 881*	8574 ± 748*	8570 ± 690*	8016 ± 565*
LVDP	36 ± 2	1 ± 1	1 ± 1	40 ± 4*	39 ± 6*	38 ± 8*	38 ± 6*	35 ± 4*

Note. Data are means ± 1 SEM ($n = 10$ each). I10 and I20, ischemia at 10 and 20 min; R1–R40, reperfusion at 1–40 min; HR, heart rate (paced, cycles/min); CPP (coronary perfusion pressure, mmHg); CVR (calculated coronary vascular resistance, mmHg/ml/min); PRP, pressure rate product (mmHg × cycles/min); LVDP (left ventricular developed pressure, mmHg) *$P < 0.05$ relative to corresponding values in vehicle-treated hearts; #$P < 0.05$ relative to corresponding values at baseline.

Creatine Kinase. There were no statistically significant differences in venous effluent concentrations of creatine kinase in the two groups of hearts under baseline control conditions. Conversely, in the 20 min of ischemia, creatine kinase rose from 42 ± 14 to 78 ± 25 units/liter ($P < 0.05$) in vehicle-treated hearts. Corresponding values in acetaminophen-treated hearts were 36 ± 8 and 44 ± 14 units/liter. By 1 min of reperfusion, creatine kinase levels had returned to or near baseline control values in both groups of hearts. From 3 to 10 min of reperfusion, creatine kinase levels were significantly lower in the presence of acetaminophen than vehicle. Although this trend was sustained from 1 to 10 min, there were no statistically significant differences between the two groups at 40 min (Fig. 3).

Chemiluminescence. During ischemia, there was a trend toward increased chemiluminescence in both groups, but this did not achieve statistical significance. From 1 to 10 min of reperfusion, the production of blue light was lower in acetaminophen-treated hearts than in the presence of vehicle ($P < 0.05$). At 3 and 6 min, the amount of blue light produced was lower than corresponding baseline levels in the presence of acetaminophen. The significant differences in the two treatment groups were less evident by 10 min of reperfusion, and were gone by 40 min (Fig. 4).

Electron Microscopy. Figures 5 through 7 illustrate electron microscopic findings in vehicle- and acetaminophen-treated hearts (top panels, acetaminophen; bottom panels, vehicle). There are no obvious differences in the two

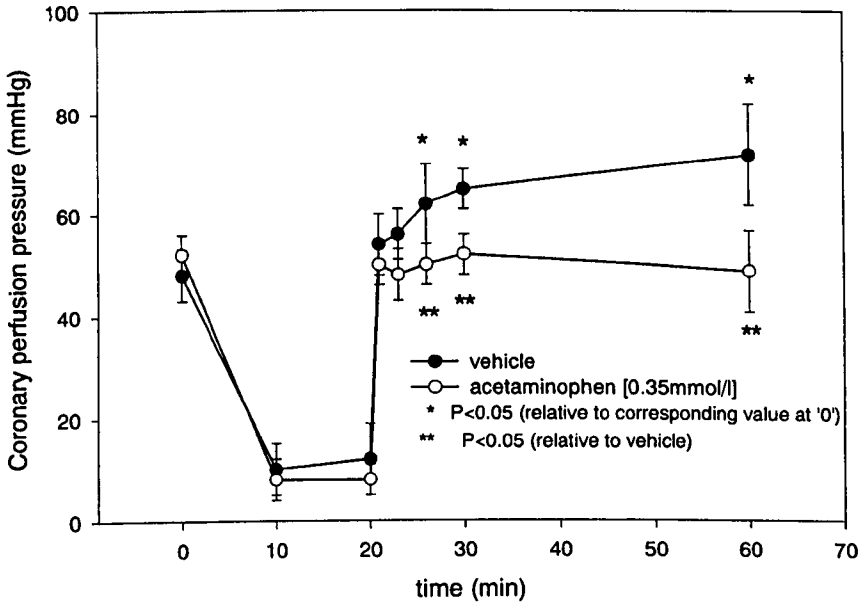

Figure 2. Coronary perfusion pressure under controlled flow conditions was another variable used to characterize cardiac function in the current investigation. Baseline (control) data were collected at 0 min. Data during ischemia were collected at 10 and 20 min, and during reperfusion at 1, 3, 6, 10, and 40 min.

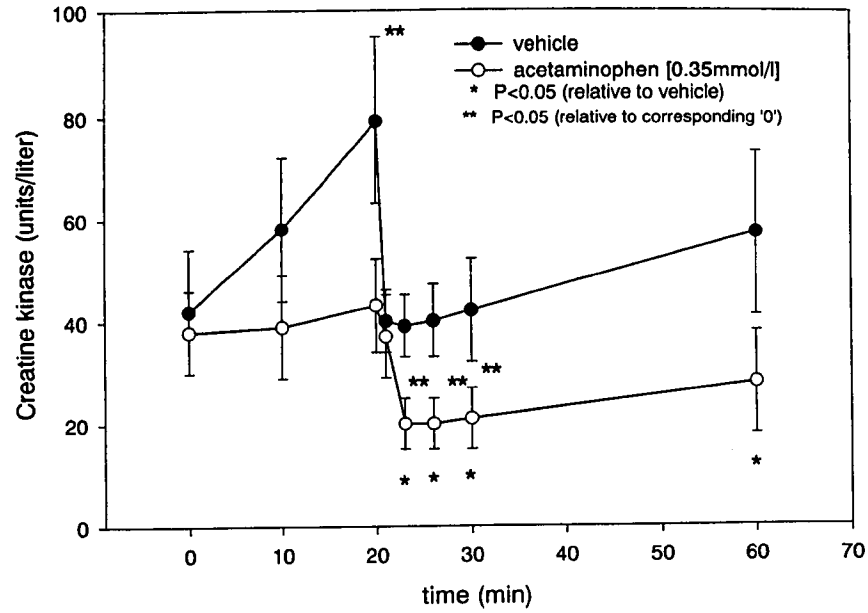

Figure 3. Creatine kinase in venous effluent samples of perfusate. Baseline (control) data were collected at 0 min. Data during ischemia were collected at 10 and 20 min, and during reperfusion at 1, 3, 6, 10, and 40 min.

groups of hearts under baseline conditions (Fig. 5). Nor could differences between vehicle and acetaminophen be easily discerned after 20 min of ischemia (Fig. 6). The most obvious differences were evident during reperfusion (Fig. 7). A close inspection of Figure 7 reveals well-defined light and dark bands with acetaminophen, but the absence of such with vehicle. Mitochondria appear dark and densely packed in acetaminophen-treated hearts, and swollen and sparsely packed in vehicle-treated hearts. The appearance of contraction bands was almost absent in the presence of acetaminophen, yet was ubiquitous with vehicle. The myofibrillar ultrastructure in acetaminophen-treated hearts during reperfusion appeared substantially the same as that during baseline control conditions. This was not true of vehicle-treated hearts.

Discussion

Water Consumption. Guinea pigs in this study tolerated the addition of 0.35 m*M* acetaminophen to their drinking water very well. Nor could we distinguish differences in taste and odor between acetaminophen-treated and regular tap water. Our values of about 80 ml/day (i.e., 25-30 ml/100g body weight/day) for water consumption by both groups are higher than those reported by Harkness and Wagner (23) of 10 ml/100g per day. We do not know the

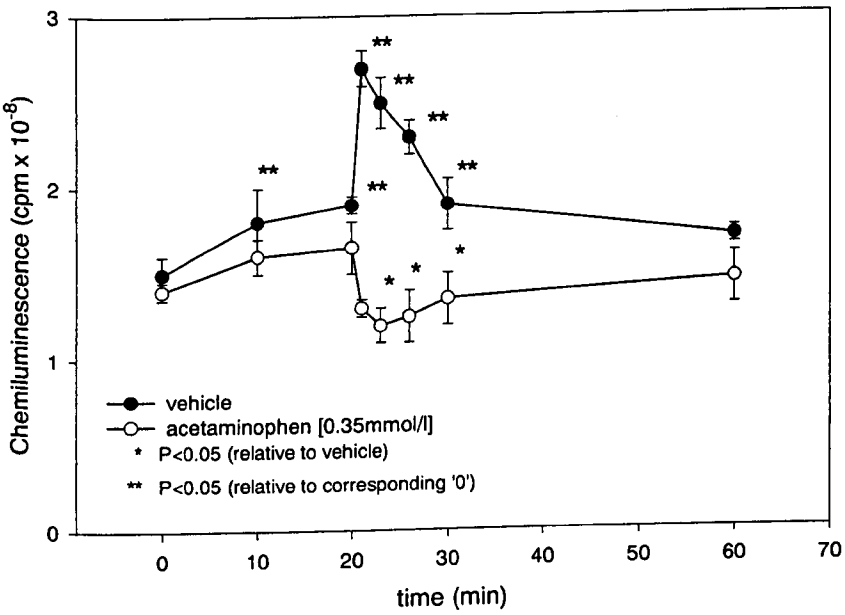

Figure 4. Chemiluminescence of venous effluent samples (an indicator of the production of peroxynitrite and/or its precursors). Baseline (control) data were collected at 0 min. Data during ischemia were collected at 10 and 20 min, and during reperfusion at 1, 3, 6, 10, and 40 min.

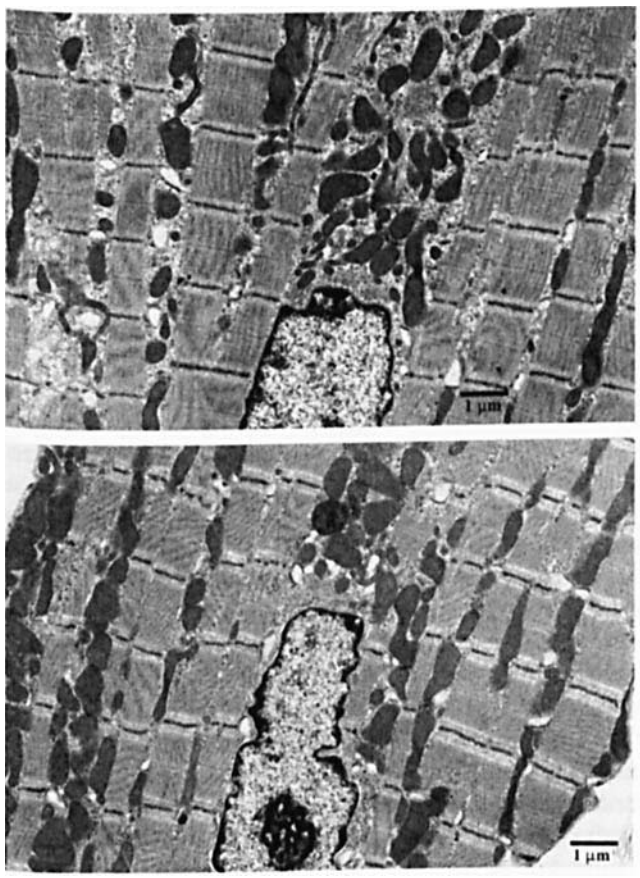

Figure 5. Electron micrograph (x10,000) of left ventricular free wall of guinea pig myocardium under baseline (control) conditions in acetaminophen-treated (top panel) and vehicle-treated animals (bottom panel). Note clarity of nuclei, mitochondria, and light/dark bands of sarcomeres.

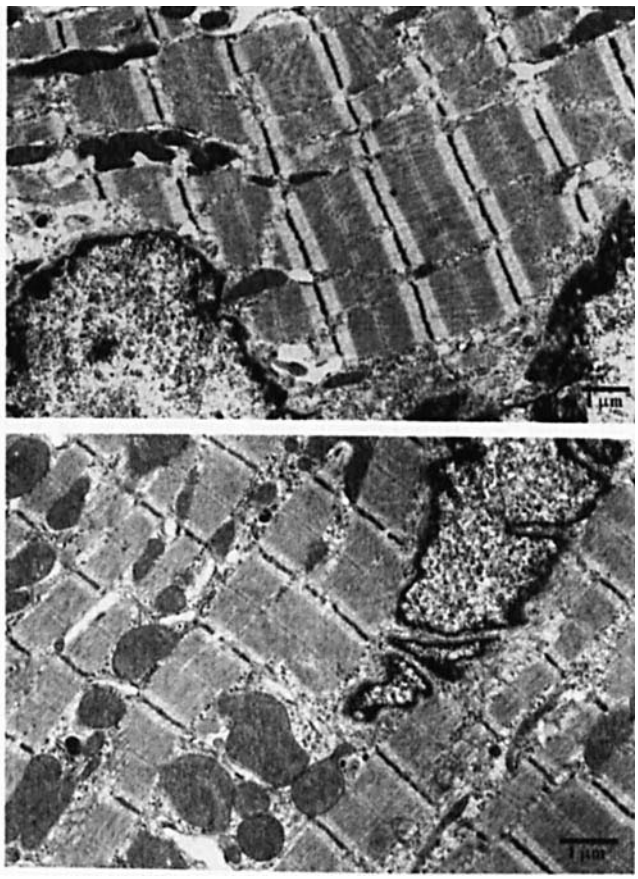

Figure 6. Electron micrograph (x10,000) of left ventricular free wall of guinea pig myocardium after 20 min of low-flow, global myocardial ischemia in acetaminophen-treated (top panel) and vehicle-treated animals (bottom panel).

Figure 7. Electron micrograph (x10,000) of left ventricular free wall of guinea pig myocardium after 20 min low-flow, global myocardial ischemia and 40 min reperfusion in an acetaminophen-treated animal (top panel) and vehicle-treated animal (bottom panel). Note multiple differences in the two panels.

specific reason(s) for this discrepancy, but it is possible that either we overestimated water consumption, or that Harkness and Wagner (23) underestimated it. Nonetheless, assuming a body water composition of approximately 60% in guinea pigs, even at the lower rate of 10 ml/100 g/day, our animals would have replaced all physiological body water with acetaminophen-containing water before 10 days

Myocardial Mechanical Function. When compared with preischemia baseline, left ventricular mechanical function in postischemic, reperfused hearts from guinea pigs administered acetaminophen daily for 10 days was not affected by a 20-min period of low-flow, global myocardial ischemia. By any current definition of reperfusion-induced myocardial failure, and considering the constraints of our experimental design (Langendorff rodent heart, crystalloid perfusate, neurogenically isolated, etc.), acetaminophen-treated hearts did not experience reperfusion-induced mechanical failure. Within 1 min of the restoration of coronary circulation postischemia, $\pm dP/dt_{max}$ and each of the other indices of mechanical function had returned to or were elevated above the baseline, preischemia control values. This was not true of vehicle-treated hearts, which routinely displayed the reported features of failure, e.g., absence of full recovery of developed pressure, $\pm dP/dt_{max}$, etc., by the end of the 40-min period of reperfusion.

Coronary Circulatory Function. Results for coronary perfusion pressure and calculated coronary vascular resistance in the two groups were consistent with the corresponding mechanical results. There were differences in the two groups of hearts as early as 3 min of reperfusion. Coronary perfusion pressure, for example, was significantly greater in vehicle-treated hearts than in acetaminophen-treated hearts from 3 to 40 min of reperfusion.

Although it was not the objective of this investigation to determine the mechanism of the unexpected finding of reperfusion-induced coronary vasoconstriction in the presence of vehicle, generally speaking, there are at least four possibilities that could have accounted for the observed response: net removal of vasodilator(s), net release of vasoconstrictor(s), incremental, edema-induced, extravascular compression, and/or a combination of these. More work is needed to sort out these possibilities and to identify the specific mechanism(s) involved.

Creatine Kinase. Acetaminophen-mediated attenuation of creatine kinase activity during reperfusion is one more indication of the cellular efficacy of acetaminophen. The reduced concentrations of effluent creatine kinase in both treatment groups during reperfusion can be explained, in part, on washout once coronary flow rates were restored. However, washout cannot explain the significant difference between treatment groups during reperfusion. The findings with creatine kinase provide further evidence that the reperfused myocardium in acetaminophen-treated animals was biochemically more intact than could be achieved in vehicle-treated animals. Such biochemical findings are consistent with both the preserved myofibrillar ultrastructure (see Electron Microscopy section below) and the improved physiological function seen in the presence of acetaminophen.

Peroxynitrite. In these experiments, the *in vitro* production of peroxynitrite most likely reflects the *in vivo* release of superoxide and nitric oxide, precursors of peroxynitrite. We cannot, with certainty, conclude that acetaminophen directly attenuates the production of peroxynitrite. We can only conclude that the production of blue light, resulting from the oxidation of luminol, is attenuated by acetaminophen. Nonetheless, these results are consistent with our earlier findings in which acetaminophen was administered acutely (4, 5). Mechanistically, however, the acetaminophen-mediated reduction in chemiluminescence is consistent with the preservation of myofibrillar ultrastructure, mechanical function, and coronary circulation. This appears to implicate peroxynitrite, and/or its metabolic precursors, in the mechanisms of postischemia, reperfusion-induced myocardial failure. Peroxynitrite and other oxidants

such as hydroxyl radical are known to play prominent roles in the dysfunction of ischemia/reperfusion injury (24, 25). For example, it is conceivable that peroxynitrite/its precursors oxidize proteins that anchor actin filaments in the sarcomeres. Such an action could disrupt the normal morphology/physiology of the sarcomere, leading to formation of contraction bands. There is a growing body of literature suggesting salutary effects of acetaminophen against oxidative tissue damage (4, 12).

Electron Microscopy. Chronic administration of acetaminophen clearly preserved myofibrillar ultrastructure in the postischemic, reperfused rodent myocardium. All of the standard indicators of a well-defined ultrastructure are consistent with this conclusion. Contraction bands were difficult to find in acetaminophen-treated myocardium, but were ubiquitous in the presence of vehicle. Mitochondria were rounded, swollen, and sparsely packed in the presence of vehicle. They were elongated, nonswollen, and densely packed in the presence of acetaminophen. Such signs are indicative of disrupted membrane structure and function, as well as aphysiological accumulation of water, and are consistent with greater cellular damage in vehicle-treated hearts than was seen with acetaminophen. The tissue damage no doubt included myocardial parenchyma as well as vascular elements. Clearly, chronically administered acetaminophen has cardioprotective properties that are revealed both as preserved myofibrillar ultrastructure, and improved physiological function in the postischemic, reperfused myocardium.

Summary and Conclusions. Biochemical, functional, and ultrastructural evidence, as reported herein, provide support for the hypothesis that the chronic administration of acetaminophen is cardioprotective against the injury caused by low-flow, global myocardial ischemia and reperfusion. Our findings reveal that the release of creatine kinase is reduced, the production of peroxynitrite/its precursors is attenuated, and postischemia, reperfusion-induced mechanical failure is less evident or absent in the presence of acetaminophen. In contrasting differences between chronic and acute administration of acetaminophen, we have noted the following: Possibly, the most evident difference is the almost immediate recovery of ventricular mechanical function upon reperfusion in the presence of chronically administered acetaminophen. Although acutely administered acetaminophen does enable impaired hearts to outperform vehicle-treated hearts during reperfusion, they have not shown the immediate and complete restoration of function that was seen in these chronically treated hearts. Subjectively, we have noted that during the period of isolation-to-stabilization, chronically treated hearts appear to achieve mechanical and electrical stability more easily and earlier than corresponding, acutely treated hearts. More work is needed to pursue this observation objectively. Finally, the current findings encourage the notion that such protective effects could extend to other pathologic conditions in the heart and to other organ systems, and should be investigated.

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