Coronary and myocardial effects of acetaminophen: protection during ischemia-reperfusion

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Merrill, G., P. McConnell, K. VanDyke, and S. Powell. Coronary and myocardial effects of acetaminophen: protection during ischemia-reperfusion. Am J Physiol Heart Circ Physiol 280: H2631–H2638, 2001.—Acetaminophen is a phenol with antioxidant properties, but little is known about its actions on the mammalian myocardium and coronary circulation. We studied isolated, perfused guinea pig hearts, and tested the hypothesis that acetaminophen-treated hearts would be protected during ischemia-reperfusion. Acetaminophen concentrations in the range of 0.3–0.6 mmol/l caused modest but significant (P < 0.05) coronary vasoconstriction and positive inotropy. The effects were more brisk during constant pressure perfusion than during constant flow. During 20 min of low-flow, global myocardial ischemia and 40 min of reperfusion, hearts treated with acetaminophen retained or recovered a greater percentage of left ventricular function than hearts treated with vehicle. Myofibrillar ultrastructure appeared to be preserved in the reperfused myocardium with acetaminophen. By using chemiluminescence and spin-trap methodologies, we investigated acetaminophen-mediated antioxidant mechanisms to help explain the cardioprotection. The burst of hydroxyl radicals seen between 0 and 10 min of reperfusion was significantly attenuated (P < 0.05) by acetaminophen but not by vehicle. The 3-morpholinosydnonimine (SIN-1) generation of peroxynitrite and its oxidative interaction with luminol to produce blue light during ischemia-reperfusion was also blocked by acetaminophen. Our results show that acetaminophen provides significant functional and structural protection to the ischemic-reperfused myocardium, and the mechanism of cardioprotection seems to involve attenuation of the production of both hydroxyl radicals and peroxynitrite.

There is limited information on the cardiovascular effects of acetaminophen, and nothing definitive on the heart and coronary circulation (6, 13). There are no reports of its influences on, i.e., myocardial ischemia-reperfusion, hypoxia-reoxygenation, or cytokine-induced tissue injury. Nakamoto et al. (18) examined the effects of acetaminophen on acute gastric mucosal injury during ischemia-reperfusion and found that it significantly reduced the total area of mucosal erosions, attenuated the increment in lipid peroxide, and blocked lipid peroxidation caused by hydroxyl radicals. They concluded that acetaminophen protects the gastric mucosa by averting the damaging effects of oxygen radicals.

Despite therapeutic use of acetaminophen for several decades (1, 22), and public perception that it has an aspirin-like ability to avert cardiovascular events (19), little is known about its potential cytoprotective properties. The study by Nakamoto et al. (18), and our preliminary observations, encouraged us to examine the effects of acetaminophen on the mammalian myocardium and coronary circulation. We tested the hypothesis that acetaminophen would be cardioprotective during myocardial ischemia-reperfusion, and that it would act via an antioxidant mechanism.

MATERIALS AND METHODS

Animals and Heart Preparation

In compliance with methods approved by an institutional review, and by following National Institutes of Health and U.S. Department of Agriculture guidelines, we obtained male and female Hartley guinea pigs (375±25 g) from Charles River Laboratories (Wilmington, MA). The guinea pig hearts were isolated, cannulated, and instrumented as previously described (31). Instrumentation included placing a flaccid latex balloon in the left ventricle (LV), attaching a pacing electrode to the base of the right ventricle, placing a large bore catheter in the trunk of the pulmonary artery (for collection of coronary venous effluent perfusate), and passing a thermistor into the right ventricle for sampling ventricular temperature (model BAT-12, Physiteemp; Clifton, NJ). Hearts

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were perfused retrogradely via the aorta without recirculation, and the balloon was inflated to yield an end-diastolic pressure of −5.0 mmHg.

Hearts were perfused either at controlled flow (Peri-Star, model 291, World Precision Instruments; Sarasota, FL) (7.0 ml/min except during low-flow ischemia when it was reduced to 1.0 ml/min) or at controlled pressure. They were allowed ~30 min postinstrumentation for monitored variables to achieve steady-state conditions. The monitored variables were the following: heart rate (HR; counts per minute paced at spontaneous rate plus 10%), LV peak systolic (LVPS, mmHg), end-diastolic pressure (mmHg), LV developed pressures (LVPD, mmHg), maximal rate of pressure increase/decrease over time (±dP/dt max) (contractility, mmHg/s), coronary perfusate flow rate (CPF; ml/min), coronary perfusion pressure (CPP; mmHg), and calculated coronary vascular resistance (CVR; mmHg·ml⁻¹·min⁻¹).

**Perfusate and Perfusion.**

The perfusate was a modified Krebs-Henseleit physiological salt solution (vehicle for acetaminophen) composed of (in mM) 128.0 NaCl, 4.7 KCl, 1.5 MgSO₄·7 H₂O, 2.5 CaCl₂, 1.2 KH₂PO₄, 24.9 NaHCO₃, 10.0 glucose, 2.0 pyruvate, and the addition of insulin (200 μU/ml), warmed to 38°C and equilibrated with 95% O₂:5% CO₂ (pH 7.40 ± 0.02). Aortic flow (antegrade coronary flow) was achieved retrogradely from a water-jacketed 1.5-l reservoir, and was continuously monitored ultrasonically (model 2N425 flowprobe and model T101 flowmeter, Transonic; Ithaca, NY).

LV pressures were monitored isovolumetrically with a pressure transducer (model P23 ID, Gould-Statham; Oxnard, CA), and inflow (coronary arterial) and outflow (pulmonary arterial, i.e., coronary venous) perfusate samples (0.5–1.0 ml) were collected anaerobically. Standard electrodes were used to measure (in mmHg) PO₂ and Pco₂, as well as pH, CO₂ content, and base excess (blood gases/pH analyzer, model 248, Bayer Diagnostics; Norwood, MA). Perfusion arterial and coronary venous oxygen contents (CaO2 and CvO2, respectively) were calculated as the products of PO₂ and the solubility of oxygen in physiological salt solution at 38°C. A solubility coefficient of $k = 2.28 \times 10^{-4}$ μl·ml⁻¹·mmHg was used (23). From these data, oxygen supply, extraction, and consumption could be calculated.

**Experimental Protocol 1.**

**Direct cardiac and coronary circulatory effects of acetaminophen.** Hearts (n = 40) in this experiment were exposed to three concentrations of acetaminophen (0.1, 0.35, and 0.6 mmol/l). They were further divided according to the mode of perfusion; i.e., controlled flow (n = 20) versus controlled pressure (n = 20). Approximately equal numbers of hearts from male and female guinea pigs were used. We selected the three concentrations to avoid levels that are toxic in humans (>$1.8–2.0$ mmol/l or $<300$ μg/ml in the plasma) but that are within the effective-to-safe range (e.g., $0.06–0.9$ mmol/l or $<10–120$ μg/ml in the plasma). Concentration response experiments also helped identify a concentration of acetaminophen that was useful in our protocols with ischemia-reperfusion.

Each heart was randomly exposed to all three concentrations of acetaminophen (with a period of washout after each addition), and data were collected only at the peak of an observed effect when monitored variables were in the steady state (i.e., ~45–60 s after initial exposure of the heart to any given concentration). The hearts were subsequently allowed ~30 min for drug washout, then similar volumes of vehicle were randomly added to the perfusate reservoir. The vehicle in all experimental protocols was Krebs-Henseleit physiological salt solution.

**Experimental Protocol 2.**

**Myofibrillar ultrastructure during ischemia-reperfusion with and without acetaminophen.** Two groups of hearts were studied in this protocol, those treated with acetaminophen (0.35 mmol/l), and those treated with vehicle. Four sets of data were collected in each group: predrug (baseline, control conditions), postdrug (20 min after exposure to drug or vehicle), ischemia (after 20 min of low-flow, global myocardial ischemia), and reperfusion (after 40 min of reflow postischemia). The hearts were exposed to acetaminophen for 80 min, and were perfused at controlled flow (7.0 ml/min) under all conditions except ischemia (1.0 ml/min). An additional group of hearts (n = 5) was exposed to 0.35 mmol/l of acetaminophen for 80 min to identify any time-dependent effects of acetaminophen. During all protocols with ischemia-reperfusion, hearts were maintained at 38°C during ischemia. This was ensured by submerging the hearts during ischemia in physiological salt solution containing the same chemical composition as that described above for perfusate. Temperature of the submersion fluid at the ventricular free walls was monitored to ensure constancy.

Before we performed electron microscopy on the hearts, the hearts were perfused with Kornovsky's fixative for 2 min at the end of predrug, postdrug, ischemia, and reperfusion conditions. The hearts were then submerged in fixative, and 20% formalin (0.35 mmol/l), and those treated with vehicle. Four sets of data were collected in each group: predrug (baseline, control conditions), postdrug (20 min after exposure to drug or vehicle), ischemia (after 20 min of low-flow, global myocardial ischemia), and reperfusion (after 40 min of reflow postischemia). The hearts were exposed to acetaminophen for 80 min, and were perfused at controlled flow (7.0 ml/min) under all conditions except ischemia (1.0 ml/min). An additional group of hearts (n = 5) was exposed to 0.35 mmol/l of acetaminophen for 80 min to identify any time-dependent effects of acetaminophen. During all protocols with ischemia-reperfusion, hearts were maintained at 38°C during ischemia. This was ensured by submerging the hearts during ischemia in physiological salt solution containing the same chemical composition as that described above for perfusate. Temperature of the submersion fluid at the ventricular free walls was monitored to ensure constancy.

Before we performed electron microscopy on the hearts, the hearts were perfused with Kornovsky's fixative for 2 min at the end of predrug, postdrug, ischemia, and reperfusion conditions. The hearts were then submerged in fixative, and 20–3 mm²) of myocardium were removed longitudinally from the posterior free wall of the LV. The blocks were postfixed with the use of 1% osmium tetroxide, followed by dehydration in graded ethanol. Samples were embedded in Epon-Araldite cocktail, sectioned with a diamond knife ultramicrotome (model LKB-20888; LKB, Sweden) and viewed with an electron microscope (model JEM-100CXII, JOEL) by using standard methods (2).

**Experimental Protocol 3.**

**Hydroxyl radical and acetaminophen.** Design of this protocol was based on the reports of Powell and Hall (20) and Powell and Tortalani (21). Predrug data were collected, followed immediately by addition of sodium salicylic acid to the perfusate reservoir (0.2 mmol/l). After the hearts had been exposed to this agent for 10 min, either acetaminophen (0.35 mmol/l) or vehicle were added to the reservoir and hearts were exposed to each agent for 20 min. The hearts were subsequently made ischemic for 20 min, followed by 40 min of reperfusion. Coronary venous effluent samples (0.5–1.0 ml) were collected during predrug conditions, after 10 min of exposure to salicylate, after 20 min of exposure to acetaminophen (vehicle), after 20 min of ischemia, and at 2, 5, 10, and 40 min of reperfusion. All samples were collected in prechilled vials and were kept on dry ice until storage at −80°C at the end of the experiment. They remained at this temperature until processed.

Because hydroxyl radical is highly unstable, it has a brief half-life. It combines with phenols yielding their hydroxylated derivatives. Our molecular trap produced a more stable half-life. It combines with phenols yielding their hydroxybenzoic acid (2,5-DHBA). 2,5-DHBA was detected according to the methods described by Powell and Hall (20). Briefly, 60 μl of filtered coronary effluent perfusate were injected into an HPLC system (Perkin-Elmer, LC series 401, 20-μl loop) equipped with a coulometric detector (Coulochem model 5100, ESA). The detection parameters were electrode 1 oxidation.
controlled pressure or controlled flow
potential, +0.4 V; electrode 2 reduction potential, −0.25 V; guard potential, −0.30 V. Chromatograms were monitored for ~10–12 min, with 2,5-DHBA curves appearing around 2 min.

Experimental Protocol 4

Peroxynitrite and acetaminophen. This protocol was based on the published reports of VanDyke and Castranova (25) and VanDyke and VanDyke (27). Hearts were treated similarly to those in experimental protocol 3, except that salicylate was not present. Acetaminophen was administered in a perfusate concentration of 0.35 mmol/l, and vehicle was given in an equal volume. Venous effluent samples (0.5–1.0 ml) were collected during predrug and postdrug conditions (20 min after initial exposure of hearts to drug), and at 2, 5, 10, and 40 min of reperfusion after the 20-min period of low-flow ischemia. Samples were treated with 100 μl of luminol (final concentration 0.6 mmol/l), 100 μl of 3-morpholinosydnonimine (SIN-1; final concentration 5.8 mmol/l), and 300 μl of physiological buffer solution. These were mixed then pipetted into 3-ml round-bottomed luminometer tubes and immediately placed in a luminometer (model LB9505C, 6-channel Berthold). The samples were analyzed for 20 min each, and the light generated was acquired, plotted, and integrated with an IBM-compatible personal computer running KINB software. The assay was reported as counts per minute integrated over the 20-min period. We also measured areas under the light curves, as well as peak heights, and time to half peak heights.

Statistical Analysis

All data were analyzed with the use of ANOVA for repeated measures. A priori tests (e.g., Tukey’s w-procedure and Fisher’s least-significant difference) were used to compare means. Statistically significant differences were established at P < 0.05. All data are means ± SE.

RESULTS

Experimental Protocol 1

Direct cardiac and coronary circulatory effects of acetaminophen. Whether coronary flow or coronary perfusion pressure was regulated, there were no significant effects of vehicle on coronary hemodynamic variables. When coronary perfusion pressure was controlled, acetaminophen at 0.35 and 0.6 mmol/l modestly but significantly (P < 0.05) reduced coronary perfusate flow rate (Table 1). Calculated coronary vascular resistance increased correspondingly, and significantly. These results were corroborated when coronary flow was controlled and coronary perfusion pressure was measured. Under these conditions, only the highest concentration of acetaminophen increased coronary perfusion pressure and coronary resistance (see Table 1). No vasodilator effects were observed.

Mechanically, vehicle had no effects on the LV, regardless of the mode of perfusion. When flow was controlled, 0.35 and 0.6 mmol/l of acetaminophen caused modest, but statistically significant, positive inotropy, e.g., LVPS increased from ~56 to 65 mmHg, and ±dP/dt max increased from ~700 to 800 mmHg/s in response to the highest concentration (Table 2). When pressure was regulated, these same concentrations increased LVPS significantly, but ±dP/dt max was significantly increased by only 0.6 mmol/l of acetaminophen.

### Table 1. Influence of acetaminophen on coronary circulation in isolated guinea pig hearts perfused at controlled pressure or controlled flow

<table>
<thead>
<tr>
<th></th>
<th>CPP, mmHg</th>
<th>CPF, ml/min</th>
<th>CVR, mmHg·ml⁻¹·min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
<td>0.35</td>
<td>0.6</td>
</tr>
<tr>
<td>CPP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh</td>
<td>50 ± 4</td>
<td>50 ± 4</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>Acet</td>
<td>50 ± 4</td>
<td>50 ± 4</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>CPF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh</td>
<td>49 ± 5</td>
<td>49 ± 5</td>
<td>49 ± 5</td>
</tr>
<tr>
<td>Acet</td>
<td>49 ± 5</td>
<td>52 ± 5*</td>
<td>49 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE. CPP, controlled coronary perfusion pressure; CPF, controlled coronary perfusate flow; CVR, calculated coronary vascular resistance; 0.1, 0.35, and 0.6, acetaminophen concentrations (mmol/l); Veh, vehicle treated; Acet, acetaminophen treated; *P < 0.05 relative to corresponding values for vehicle.

### Table 2. Influence of acetaminophen on left ventricular function in isolated guinea pig hearts perfused at controlled pressure or controlled flow

<table>
<thead>
<tr>
<th></th>
<th>LVPS, mmHg</th>
<th>+dP/dt max, mmHg/s</th>
<th>−dP/dt max, mmHg/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
<td>0.35</td>
<td>0.6</td>
</tr>
<tr>
<td>CPP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VT</td>
<td>59 ± 6</td>
<td>59 ± 6</td>
<td>61 ± 7</td>
</tr>
<tr>
<td>AT</td>
<td>60 ± 5</td>
<td>67 ± 5*</td>
<td>90 ± 6*</td>
</tr>
<tr>
<td>CPF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VT</td>
<td>56 ± 3</td>
<td>56 ± 3</td>
<td>58 ± 3</td>
</tr>
<tr>
<td>AT</td>
<td>56 ± 3</td>
<td>59 ± 2*</td>
<td>65 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVPS, left ventricular peak systolic pressure; ±dP/dt max, rates of change of left ventricular pressures (contractility) over time. *P < 0.05 relative to corresponding values for vehicle.
In this protocol there were no significant effects of acetaminophen on any other monitored variable. HPLC analysis of perfusate collected from the reservoir, the arterial circuit (several centimeters upstream to the heart), and the coronary venous effluent (pulmonary artery) revealed circulating concentrations of acetaminophen at a final concentration of 0.35 mmol/l in the perfusate reservoir.

**Experimental Protocol 2**

Myofibrillar ultrastructure and ventricular function during ischemia-reperfusion with and without acetaminophen. During 20 min of low-flow ischemia, \( \frac{dP}{dt_{\text{max}}} \) was significantly greater (\( P < 0.05 \)) in the presence of 0.35 mmol/l of acetaminophen than in its absence. After 20–40 min of reperfusion, the maximal rate of pressure increase over time (\( \frac{dP}{dt_{\text{max}}} \)) recovered 100% of its predrug/postdrug value (Fig. 1). LVPD

![Fig. 1](image1.png)

**Fig. 1.** Influence of acetaminophen and vehicle on maximal rate of pressure increase over time (\( \frac{dP}{dt_{\text{max}}} \)) during predrug/postdrug (−20 and 0 min, respectively), ischemia (10 and 20 min), and after 20 and 40 min or reperfusion (40 and 60 min, respectively).

H2634 MECHANISM OF APAP CARDIOPROTECTION

![Fig. 2](image2.png)

**Fig. 2.** Influence of acetaminophen and vehicle on left ventricular (LV) developed pressure (LVPD) under the four conditions specified in the ischemia-reperfusion protocol.

![Fig. 3](image3.png)

**Fig. 3.** Electron micrograph (×8,000, reduced to one-fourth original size) of LV myocardium under predrug conditions (A), during reperfusion in the absence (B), and presence (C) of acetaminophen. Note diffuse, blurred Z lines, presence of contraction bands, and swollen, sparsely packed mitochondria in B relative to A and C.
showed patterns similar to those for $+\mathrm{d}P/\mathrm{d}t_{\text{max}}$ (Fig. 2). Vehicle-treated hearts recovered ~65–70% of their corresponding values. LV end-diastolic pressure was elevated during reperfusion in vehicle-treated hearts (15 ± 5 mmHg at 40 min) and was significantly greater than corresponding values in the presence of acetaminophen (5 ± 2 mmHg at 40 min, $P < 0.05$).

Acetaminophen appeared to preserve myocardial ultrastructure during reperfusion. Fig. 3 illustrates myocardial ultrastructure under predrug baseline conditions and after 40 min of reperfusion in the absence and presence of 0.35 mmol/l of acetaminophen. Note the multiple contraction bands and swollen mitochondria in the vehicle-treated hearts, and their absence with acetaminophen. Selected morphological data were compared semiquantitatively with the use of planimetry. For example, under baseline conditions, mitochondria (nonswollen) occupied ~32 ± 7% of the total surface area (cm²) of the electron micrographs. This did not change significantly after 40 min of reperfusion in the presence of acetaminophen. Conversely, in vehicle-treated hearts, the swollen, sparsely packed mitochondria occupied 67 ± 13% of the total surface area ($P < 0.05$).

**Experimental Protocol 3**

**Hydroxyl radical and acetaminophen.** Metabolically (perfusate gases, pH, base excess) vehicle- and acetaminophen-treated hearts did not differ significantly (Table 3). All values were within the normal ranges for isolated rodent hearts perfused with physiological salt solution.

Whether reported as the absolute rate of release (pmol/min), or as a percentage of the initial rate of release, the production of 2,5-DHBA in the early minutes of reperfusion was significantly attenuated by 0.35 mmol/l of acetaminophen. For example, at ~2 min of reperfusion, production of 2,5-DHBA in the presence of acetaminophen was only about one-half that seen with vehicle (Fig. 4). These differences persisted through 10 min of reperfusion but were absent by 40 min.

**Table 3. Metabolic status of perfused hearts during ischemia and reperfusion in the absence and presence of acetaminophen**

<table>
<thead>
<tr>
<th>Postdrug</th>
<th>Arterial</th>
<th>Venous</th>
<th>Ischemia</th>
<th>Arterial</th>
<th>Venous</th>
<th>Reperfusion</th>
<th>Arterial</th>
<th>Venous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P_{O_2}$ mmHg</td>
<td>$P_{CO_2}$ mmHg</td>
<td>pH units</td>
<td>Base Excess mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acet</td>
<td>609 ± 9</td>
<td>38 ± 2</td>
<td>7.38 ± 0.02</td>
<td>-4.5 ± 0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh</td>
<td>620 ± 12</td>
<td>34 ± 1</td>
<td>7.40 ± 0.01</td>
<td>-5.2 ± 0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acet</td>
<td>82 ± 11</td>
<td>55 ± 4</td>
<td>7.17 ± 0.01</td>
<td>-6.3 ± 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veh</td>
<td>75 ± 11</td>
<td>49 ± 2</td>
<td>7.24 ± 0.02</td>
<td>-5.3 ± 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. $P_{O_2}$ and $P_{CO_2}$, partial pressures of oxygen and carbon dioxide, respectively; Acet, acetaminophen (0.35 mmol/l); Veh, vehicle. There are no statistically significant differences between any sets of numbers in corresponding columns of Acet/Veh data. There are numerous differences within columns (e.g., venous $P_{CO_2}$ ischemia vs. postdrug).

Experimental Protocol 4

**Peroxynitrite and acetaminophen.** There were marked and statistically significant differences in the production of blue light (chemiluminescence) between vehicle- and acetaminophen-treated hearts. For example, during 20 min of ischemia, and in the early minutes of reperfusion, chemiluminescence was abolished by 0.35 mmol/l of acetaminophen (Fig. 5). This difference persisted through 10 min of reperfusion but was gone by 40 min. The difference was evident whether data were evaluated as peak counts per minute, integrated counts per minute, or cross-sectional area beneath luminescence curves (Table 4). When expressed as time for the blue light to reach its zenith (in minutes), acetaminophen-treated hearts took significantly longer than vehicle-treated hearts (e.g., 18 vs. 16 min, respectively, $P < 0.05$).

Fig. 4. Changes in coronary venous effluent concentrations of 2,5-dihydroxybenzoic acid (2,5-DHBA), as a function of time of perfusion, in the absence and presence of 0.35 mmol/l acetaminophen (therapeutic dose) expressed as a percentage of the baseline control values. Note: ~20 min (10 min postadministration of salicylate); ~10 min (20 min postadministration of acetaminophen); 0 min (after 20 min ischemia); 0–40 min (during reperfusion).
these hearts were exposed to circulating concentrations of acetaminophen equal to 50 μg/ml. This is inside the range of therapeutic-to-safe concentrations described in the literature.

Comparing results using controlled flow versus controlled pressure, a picture of modest but significant acetaminophen-mediated coronary vasoconstriction emerged. However, only the highest concentration (0.6 mmol/l; ~100 μg/ml in human plasma) consistently produced this effect, and there were qualitative differences between the two modes of circulation. Our objective for this part of the study was to identify coronary vasoactive properties of acetaminophen. From these results, we cannot determine the mechanism of acetaminophen-mediated coronary vasoconstriction, nor if the effects were mediated directly at the vascular smooth muscle or at the underlying endothelium. We can only conclude that concentrations at the lower end of the safe-to-therapeutic range did not directly affect the coronary circulation, and those that did produced only modest, albeit statistically significant effects. To our knowledge, no one has reported the influence of acetaminophen at 50 μg/ml on the human heart and coronary circulation.

At the highest concentration acetaminophen appears to have positive inotropic properties. Although this effect was influenced by the mode of circulation, the mechanical responses were not caused by the circulatory effects of the drug. If the circulatory effects had influenced mechanics, one would have expected negative inotropy in the face of a reduction in oxygen supply, i.e., acetaminophen-mediated coronary vasoconstriction. Conversely, LVDP in some isolated, perfused rodent heart preparations seems to be sensitive to coronary perfusion pressure. Hence, under conditions of controlled flow in such preparations, small- to moderate-increments in coronary perfusion pressure can indirectly cause positive inotropy.

Acetaminophen and Myofibrillar Ultrastructure During Ischemia-Reperfusion

Ventricular function appeared to be protected during ischemia-reperfusion in the presence of 0.35 mmol/l of acetaminophen (e.g., values >10 mmHg for end-diastolic pressure were rarely seen during reperfusion). Conversely, during the early minutes of reperfusion, LV end-diastolic pressure was often elevated to values of 20–30 mmHg with vehicle (although these declined

### DISCUSSION

**Choice of Langendorff Heart Preparation**

One of the objectives of the current investigation was to determine if acetaminophen acts directly on the coronary circulation and myocardium. The authors chose the isolated, perfused, guinea pig heart preparation developed by Bunger et al. (7, 8) because the coronary circulation of this preparation has in vivo-like characteristics (e.g., it autoregulates). The authors are not aware of any other rodent heart, Langendorff preparations, including those of the rat and rabbit, which possess this physiological quality (9, 14, 24). Also, our techniques of isolating, arresting, and instrumenting the guinea pig heart in situ ensure that the coronary circulation is not embolized with air before the onset of an experimental protocol. The commonly used practice of excising hearts and then plunging them into a chilled buffer solution to induce cardiac arrest cannot ensure freedom from air embolization and the subsequent complications that accompany it.

**Direct Effect of Acetaminophen on Coronary Circulation and Myocardium**

To the authors’ knowledge, this is the first published report aimed at determining if acetaminophen has direct cardiac and/or coronary circulatory effects in the mammalian myocardium. In identifying these actions, two of our main concerns were to avoid concentrations that are toxic in humans (>300 μg/ml), i.e., to stay within the safe-to-therapeutic range (i.e., 10–120 μg/ml) (10, 12), and to identify effects that were not complicated by blood-borne elements and neurohormons (an impossibility when using whole blood as perfusate, and when studying the heart in situ). At a final concentration of 0.35 mmol/l in the perfusate reservoir,
towards more physiological values by 20–40 min of reperfusion). Developed pressure and contractility recovered only sluggishly during the same time interval. It is noteworthy that in the absence of acetaminophen, myofibrillar ultrastructure was lost and never redefined during reperfusion. This was most evident in the marked reduction in the density of Z lines (suggesting a loss of protein content), the abundance of contraction bands, and the ubiquitous swelling of mitochondria. Collectively, these observations suggest accumulation of intracellular water, with the accompanying congestion that produces LV diastolic pressures of 20–30 mmHg. Thus both physiological and morphological analyses point to acetaminophen-mediated preservation of structure and function in the postsischemic, reperfused myocardium, i.e., cardioprotection.

Oxidants, Ischemia-Reperfusion, and Acetaminophen

Reperfusion is essential to salvage of the postsischemic myocardium, but can cause additional tissue damage (5, 16). Increasing evidence suggests that oxidants such as hydroxyl radical, $O_2^*$, and peroxynitrite play prominent roles in mediating myocardial dysfunction during reperfusion (15), and with administration of cytokines (11). Such injury can be manifested as disrupted ultrastructure, decreased mechanical function, impaired coronary circulation, and reperfusion arrhythmias.

Acetaminophen is a phenol that possesses little anti-inflammatory behavior (22). Consequently, it has been underinvestigated relative to its potential for cytoprotective properties. Phenols are strong antioxidants that are commonly found in a variety of prescription and nonprescription drugs (26, 28). Therefore, it is conceivable that they might confer preservation or protection of organs in damaging conditions, such as ischemia-reperfusion, hypoxia-reoxygenation, and cytokine-induced tissue injury. These possibilities, and their mechanistic relations to the production and release of oxygen radicals, require further investigation.

Hydroxyl Radical and Acetaminophen

Bolli et al. (3, 4) found that administration of antioxidants at the onset of reperfusion reduced the generation/release of hydroxyl radicals. Powell and Hall (20) reported that hydroxyl radical was an important contributor to tissue injury in the early minutes of reperfusion in rat hearts, and McHugh et al. (17) observed that estrogen reduces the production of hydroxyl radicals during the early minutes of reperfusion in the canine myocardium.

In the present study, vehicle-treated hearts displayed significant production and release of hydroxyl radicals during the first few minutes of reperfusion after 20 min of global low-flow ischemia. This early burst of hydroxyl radicals was attenuated by acetaminophen. Such an effect is in keeping with the known antioxidant properties of the drug, and to the authors’ knowledge, is the first time it has been reported in the mammalian myocardium. Whether acetaminophen acted by scavenging hydroxyl radical or by reducing its production cannot be determined from our experiments. We can only conclude that its phenolic properties enabled it to behave as an effective antioxidant in the reperfused, stunned myocardium, and that these actions translated immediately into preservation of ultrastructure and improved mechanical function.

Peroxynitrite and Acetaminophen

The antioxidant ability of acetaminophen to attenuate production of hydroxyl radicals was corroborated by an apparent ability to block the oxidative actions of peroxynitrite, i.e., the oxidation of luminol by peroxynitrite to produce blue light. Peroxynitrite is generated from the simultaneous production of NO (via SIN-1) and $O_2^*$ (26, 28). The effects of acetaminophen on peroxynitrite were most evident during the early minutes of reperfusion. This suggests that the temporal course of oxidative damage by peroxynitrite, at least in our preparation during low-flow, global myocardial ischemia, occurs early on in reperfusion.

In conclusion, acetaminophen has modest, but significant, concentration-dependent effects on the heart and coronary circulation. It displays cardioprotective properties against dysfunction and injury caused by transient, low-flow, myocardial ischemia-reperfusion. The mechanisms of cardioprotection seem to involve attenuation of the actions of hydroxyl radicals and peroxynitrite anions. Both are implicated in myocardial injury and dysfunction (30, 32), although the extent of involvement might be influenced by the perfusion medium (29).

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