Acetaminophen and low-flow myocardial ischemia: efficacy and antioxidant mechanisms

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Merrill, Gary F. Acetaminophen and low-flow myocardial ischemia: efficacy and antioxidant mechanisms. Am J Physiol Heart Circ Physiol 282: H1341–H1349, 2002; 10.1152/ajpheart.00716.2001.—In the current study, the cardioprotective efficacy of 0.35 mmol/l acetaminophen administered 10 min after the onset of a 20-min period of global, low-flow myocardial ischemia was investigated. Matched control hearts were administered an equal volume of Krebs-Henseleit physiological buffer solution (vehicle). In separate groups of hearts, the concentration-dependent, negative inotropic properties of hydrogen peroxide and the ability of acetaminophen to attenuate these actions, as well as the effects of acetaminophen on ischemia-reperfusion-mediated protein oxidation, were studied. Acetaminophen-treated hearts regained a significantly greater fraction of baseline, preischemia control function during reperfusion than vehicle-treated hearts. For example, contractility [rate of maximal developed pressure in the left ventricle (±dP/dt max)] after 10 min of reperfusion was 109 ± 24 and 42 ± 9 mmHg/s (P < 0.05), respectively, in the two groups. The corresponding pressure-rate products were 1,840 ± 434 vs. 588 ± 169 mmHg-beats·min⁻¹ (P < 0.05). Acetaminophen attenuated peroxynitrite-mediated chemiluminescence in the early minutes of reperfusion (e.g., at 6 min, corresponding values for peak light production were −8 × 10⁶ counts/min for vehicle vs. −4 × 10⁶ counts/min for acetaminophen, P < 0.05) and the negative inotropic effects of exogenously administered hydrogen peroxide (e.g., at 0.4 mmol/l hydrogen peroxide, pressure-rate products were −1.0 × 10⁴ and 3.8 × 10³ mmHg-beats·min⁻¹ in acetaminophen- and vehicle-treated hearts, respectively, P < 0.05). Ischemia-mediated protein oxidation was reduced by acetaminophen. The ability of acetaminophen to attenuate the damaging effects of peroxynitrite and hydrogen peroxide and to limit protein oxidation suggest antioxidant mechanisms are responsible for its cardioprotective properties during postischemia-reperfusion.

coronary circulation; ventricular function; peroxynitrite; hydrogen peroxide

ACETAMINOPHEN WAS INTRODUCED into Western medicine more than 100 years ago, and its pain-relieving and temperature-lowering actions have been under investigation for several decades (1, 9, 26). Potential cardiovascular properties of acetaminophen have gone undiscovered, in part, because no one has made an effort to do the experiments. This might have been influenced by standard textbooks of pharmacology that report that acetaminophen lacks efficacy in the cardiovascular system of mammals (12). The recent literature, however, suggests that investigators are beginning to fill this void. For example, Nakamoto et al. (22) reported beneficial effects of acetaminophen against gastric mucosal injury caused by ischemia-reperfusion in the rat. Farquhar et al. (10) reported reduced renal dysfunction in the stressed human kidney in the presence of acetaminophen vs. ibuprofen, and Colletti et al. (7) found that ibuprofen caused significantly greater renal arterial vasoconstriction than acetaminophen in sodium-depleted dogs.

One purpose of the current investigation was to determine whether administration of acetaminophen after the onset of low-flow myocardial ischemia could produce cardioprotection during the subsequent period of reperfusion. Another purpose was to investigate several antioxidant mechanisms that might help explain the cardioprotective properties of acetaminophen, e.g., does acetaminophen attenuate the negative inotropic actions of exogenously administered hydrogen peroxide? The latter objective seemed important because it is well known that hydrogen peroxide releases hydroxyl radical via the Fenton reaction (2, 14), and it has already been shown that acetaminophen attenuates the production of hydroxyl radical in the posts ischemic-reperfused myocardium (21).

MATERIALS AND METHODS

Animals and Heart Preparation

In adherence with National Institutes of Health/United States Department of Agriculture guidelines and after institutional review and approval, Hartley strain guinea pigs of both genders weighing 375 ± 25 g were obtained from Charles River Laboratories (Wilmington, MA). They were allowed several days to acclimate to the new housing conditions and were brought to the laboratory and euthanized by cranial crushing. Hearts were isolated, cannulated, and instrumented in situ as originally described by Bunger et al. (4, 5) and modified by Wei et al. (34). Instrumentation included inserting a flaccid latex balloon into the left atrium and advancing it across the mitral valve and into the left ventricle, where it was filled with Krebs-Henseleit buffer (KHB) solution to an end-diastolic pressure of 0–5 mmHg (volume of
75–100 μl. A pacing electrode was attached to the base of the right ventricle, and a large-bore polyethylene (PE) cannula (PE-240) was inserted into the trunk of the pulmonary artery for collection of samples of coronary venous effluent perfusate. Normothermic heart temperature was confirmed by passing a thermistor probe into the right ventricle (model BAT-12, Physitemp; Clifton, NJ). Hearts were perfused retrogradely via the cannulated aorta without recirculation.

Upon completion of the instrumentation, hearts were perfused in increments of ~2 ml/min at 3- to 4-min intervals until a control rate of 7–8 ml/min was reached. Flow was controlled at this rate throughout the experiments except during low-flow ischemia, when it was reduced to 1 ml/min (model 291, Peri-Star pump, World Precision Instruments; Sarasota, FL). Hearts were allowed 30 min of postinstrumentation for monitored variables to achieve steady-state conditions. Monitored variables included the following: heart rate (HR; paced at a spontaneous rate plus 10%, in beats/min), left ventricular developed pressure (LVDP; in mmHg, the difference between peak systolic and end-diastolic pressures in the left ventricle), pressure-rate product (PRP; i.e., LVDP × HR), rate of maximal developed pressure (±dP/dt max; in mmHg/s, contractility), coronary perfuse flow rate (CPF; in ml/min), coronary perfusion pressure (CPP; in mmHg), and calculated coronary vascular resistance (CVR; in mmHg·ml⁻¹·min⁻¹).

**Perfusate and Perfusion Modality**

Perfusate was a modified KHB physiological solution (vehicle for acetaminophen) containing (in mmol/l) 128.0 NaCl, 4.7 KCl, 1.5 MgSO₄·7H₂O, 2.5 CaCl₂, 1.2 KH₂PO₄, 24.9 NaHCO₃, 10.0 glucose, and 2.0 pyruvate and 200.0 KCl, 1.5 MgSO₄ (vehicle for exogenously administered hydrogen peroxide). Hearts were allowed 30 min of postinstrumentation for monitoring of pH and perfusate gases (0.5 ml arterial and 1.0 ml venous) and oxygen consumption could be calculated.

**Experimental Protocols**

Four experimental protocols were completed. One protocol was to rule out time-dependent changes with acetaminophen. A second protocol was to determine the effects of acetaminophen on cardiac function and on peroxynitrite-mediated chemiluminescence during ischemia-reperfusion. The third protocol examined the effects of acetaminophen on the cardiac actions of exogenously administered hydrogen peroxide. A fourth experiment tested the effects of acetaminophen on ischemia-reperfusion-mediated protein oxidation. Details of each are given below.

**Time-dependent actions of acetaminophen and vehicle.** All experiments in this investigation were conducted in either vehicle- or acetaminophen-treated hearts. In our laboratory, an average experiment in perfused guinea pig hearts lasted 90–120 min. After extraction and instrumentation, hearts in this protocol were allowed 30 min for monitored variables to achieve steady-state conditions. A sample of n = 10 hearts was used, 5 hearts each treated with vehicle or acetaminophen (0.35 mmol/l) immediately after collection of baseline, control data at the end of the 30-min stabilization period. Cardiac function, perfuse gases, pH, and coronary circulation were monitored at 15-min intervals during the 90-min experimental period.

**Hydrogen peroxide and acetaminophen.** A sample of n = 20 hearts was used in this experiment. In preliminary experiments (not included in the n = 20 hearts), we worked out a concentration-response profile for exogenously administered hydrogen peroxide. After baseline, control data were collected, either vehicle (n = 10 hearts) or acetaminophen (0.35 mmol/l; n = 10 hearts) was added to the perfusate reservoir. Hearts were allowed 20 min of exposure to either agent. Subsequently calculated volumes of a stock solution of hydrogen peroxide were added incrementally to the perfusate to yield final concentrations of 0.1, 0.25, and 0.4 mmol/l hydrogen peroxide. Hearts were allowed 15 min of exposure to each concentration before the next addition was made. Data were collected at the end of each of the 15-min periods for subsequent comparison.

**Ischemia-reperfusion, peroxynitrite, and acetaminophen.** The objective of this experiment was to determine whether administration of acetaminophen after 10 min of ischemia could produce beneficial, cardioprotective effects during the subsequent period of reperfusion. We also wanted to test the effects of acetaminophen on peroxynitrite-mediated oxidation of luminol, i.e., on the ability of effluent perfusate samples to produce chemiluminescence. A sample of n = 20 hearts was used. Ten hearts were treated with vehicle, and ten hearts were treated with acetaminophen (0.35 mmol/l). After collection of baseline, control data, hearts were submerged in physiologic KHB (vehicle) at 38 °C, and CPF was immediately reduced from 7 to 8 ml/min to produce lowflow, global myocardial ischemia. Normothermia was maintained throughout the period of ischemia to eliminate the confounding effects that result from hypothermic perfusion of the ischemic myocardium (i.e., as flow is reduced, the myocardial temperature can drop from 38 to <30 °C over the course of 20 min, personal observations). During ischemia, data were collected at 5-min intervals. Subsequently, flow was restored to 7–8 ml/min, hearts were removed from the submersion fluid, i.e., the heart chamber was drained, and hearts were reperfused for 40 min. During reperfusion, data were collected at 10-min intervals.

Twenty additional hearts were divided into four groups of five hearts each as follows: vehicle treated (n = 5), acetaminophen treated (0.35 mmol/l; n = 5), urate treated (0.25 mmol/l; n = 5), and acetaminophen and urate-treated (n = 5). Urate was used to scavenge peroxynitrite to aid in the identification of nonspecific chemiluminescence that might have been due to sources other than peroxynitrite.

**Retrieval of data included collection of coronary venous samples of perfusate (1 ml each) in all hearts under baseline, control conditions, at 10 and 20 min of ischemia, and at 1, 3, 6, 10, and 40 min of reperfusion. Samples were collected in prechilled vials and stored at −80 °C for subsequent analysis of peroxynitrite and chemiluminescence.** Peroxynitrite and chemiluminescence were analyzed following the published procedures of VanDyke et al. (30, 31) and Merrill et al. (21).

**Ischemia-reperfusion, protein oxidation, and acetaminophen.** One of the signs of protein damage caused by ischemia-reperfusion injury in the mammalian myocardium is the
presence of increased carbonyls in the tissue. To investigate the influence of acetaminophen on ischemia-reperfusion-mediated protein damage, two groups of hearts were used: a vehicle-treated group (n = 12) and an acetaminophen-treated group (n = 12). A protocol similar to that described above for experiment 3 was used. In this experiment, however, tissue samples of the myocardium had to be collected at each of the experimental endpoints, i.e., control, baseline conditions, at the end of ischemia, and after 40 min of reperfusion. At each of these times, ventricles were quickly excised from the perfusion apparatus, blotted dry, and plunged into liquid nitrogen.

Protein oxidation, i.e., carbonyl contents of the myocardium, was analyzed according to the published procedures of Schacter et al. (28). Briefly, ventricular tissue was homogenized in HEPES suspension buffer containing protease inhibitors and then centrifuged to obtain the soluble fraction. Protein carbonyls were analyzed using a commercially available kit (OxyBlot, Intergen; Purchase, NY). Carbonyl groups on soluble cellular proteins were derivatized and then bound onto Zeta-Probe membranes using the Bio-Dot SF Microfiltration Apparatus (Bio-Rad Laboratories; Hercules, CA). The membrane-bound proteins were then blocked and probed with primary and secondary antibodies in blocking buffer. The developed membranes were digitized and analyzed using computer-assisted densitometry (SigmaScan, Jandel Scientific; Chicago, IL).

**Statistical Analysis**

All data were analyzed using analysis of variance 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, and all data are reported as means ± SE.

**RESULTS**

**Time-Dependent Actions of Acetaminophen and Vehicle**

There were no statistically significant differences in any recorded variables between vehicle- and acetaminophen-treated hearts in this experiment (Table 1). Hearts were paced and on the average displayed rates of 270 ± 20 and 264 ± 16 beats/min in the two groups, respectively. There was some variability in HR (data not shown), but these values are consistent with previous data collected in our laboratory and elsewhere. Indexes of ventricular function, e.g., ±dP/dt max, PRP, and LVDP, remained constant throughout the 90-min experimental period and did not vary in the two groups. However, there was a trend toward time-dependent improvement in ventricular function with acetaminophen that was not evident in vehicle-treated hearts, but this did not achieve significance (Table 1).

Ventricular wet weights were recorded at the end of each experiment in both groups. There were no differences, suggesting that whatever edema might have occurred with time was similar in both groups.

**Hydrogen Peroxide and Acetaminophen**

We chose the concentrations of 0.1, 0.25, and 0.4 mmol/l hydrogen peroxide because in preliminary experiments we found that concentrations up to and including 0.1 mmol/l had no effect on the myocardium. A concentration of 0.25 mmol/l consistently produced recordable negative inotropic effects, and 0.4 mmol/l produced marked, statistically significant, negative inotropism. These concentrations are also consistent with recent studies done by others (3, 6, 8, 14) in the rodent myocardium. After several minutes, concentrations >0.4 mmol/l regularly produced contracture, cardiac arrest, and ventricular fibrillation.

The consistently most prominent effect of hydrogen peroxide was concentration-dependent negative inotropy (Table 2). This effect was significantly attenuated by acetaminophen. For example, all three indexes of ventricular function were consistently reduced by 50–75% in vehicle-treated hearts by the highest concentration of hydrogen peroxide. Conversely, in acetaminophen-treated hearts, on the average, the highest concentrations of hydrogen peroxide rarely reduced function by >25% (Table 2). Figure 1 is a representative

<table>
<thead>
<tr>
<th>Table 1. Influence of acetaminophen and vehicle on heart rate and hemodynamic variables as a function of time</th>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
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<tr>
<td></td>
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<tr>
<td><strong>CPP, mmHg</strong></td>
</tr>
<tr>
<td>Acetaminophen</td>
</tr>
<tr>
<td>Vehicle</td>
</tr>
<tr>
<td><strong>CVR, mmHg·ml⁻¹·min⁻¹</strong></td>
</tr>
<tr>
<td>Acetaminophen</td>
</tr>
<tr>
<td>Vehicle</td>
</tr>
<tr>
<td><strong>+dP/dt max, mmHg/s</strong></td>
</tr>
<tr>
<td>Acetaminophen</td>
</tr>
<tr>
<td>Vehicle</td>
</tr>
<tr>
<td><strong>PRP, mmHg/min</strong></td>
</tr>
<tr>
<td>Acetaminophen</td>
</tr>
<tr>
<td>Vehicle</td>
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</tbody>
</table>

Data are means ± SE; n = 10 guinea pigs. Baseline, predrug control conditions. CPP, coronary perfusion pressure; CVR, calculated coronary vascular resistance; +dP/dt max, change in left ventricular pressure with change in time (contractility); PRP, pressure-rate product (product of heart rate and left ventricular developed pressure (LVDP)).
sample of the effects of 0.4 mmol/l hydrogen peroxide on function and hemodynamic variables in the presence of acetaminophen and vehicle. Note the prominent and developing cardiac contracture in the presence of vehicle and the evidence of reduced contractility.

Ischemia-Reperfusion, Peroxynitrite, and Acetaminophen

When administered midway through a 20-min period of ischemia, acetaminophen significantly preserved ventricular function during the subsequent period of reperfusion. For example, in acetaminophen-treated hearts, $\pm dP/dt_{max}$ was restored, on the average, to ~50% of its baseline, preischemia value throughout the 40-min period of reperfusion. Conversely, vehicle-treated hearts rarely recovered >20–25% of their baseline function during the same time interval. Figure 2 illustrates this in one vehicle-treated and one acetaminophen-treated heart. A similar picture emerged for other indexes of function, e.g., developed pressure, PRP, and $\pm dP/dt_{max}$ (Fig. 3). These physiological, functional differences were consistent with morphological changes seen under the two conditions. For example, electronmicrographs taken of the left ventricular free wall under baseline, control conditions and after 20 min of ischemia plus 40 min of reperfusion in the presence of vehicle and acetaminophen demonstrated the ability of acetaminophen to preserve left ventricular myofibrillar ultrastructure (Fig. 4, A–C).

During reperfusion, peroxynitrite-mediated oxidation of luminol (chemiluminescence) was significantly

### Table 2. Influence of hydrogen peroxide on ventricular function in the absence (vehicle) and presence of acetaminophen

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hydrogen Peroxide, mmol/l</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>$+dP/dt_{max}$, mmHg/s</td>
<td>540 ± 64</td>
<td>540 ± 64</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>518 ± 48</td>
<td>528 ± 52</td>
</tr>
<tr>
<td>$-dP/dt_{max}$, mmHg/s</td>
<td>480 ± 48</td>
<td>488 ± 48</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>504 ± 48</td>
<td>500 ± 42</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>54 ± 6</td>
<td>58 ± 6</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>52 ± 4</td>
<td>53 ± 5</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRP, mmHg/min</td>
<td>14,640 ± 890</td>
<td>14,640 ± 800</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>14,320 ± 818</td>
<td>14,388 ± 888</td>
</tr>
</tbody>
</table>

Data are means ± SE; $n = 8$ guinea pigs. $-dP/dt_{max}$, decrease in LV pressure with change in time (contractility). $*P < 0.05$ relative to corresponding values for baseline and acetaminophen.
attenuated by acetaminophen (Fig. 5). When hearts were treated with urate with or without acetaminophen, luminol-mediated chemiluminescence relative to vehicle-treated hearts was abolished, i.e., urate produced the same effect as acetaminophen (Table 3).

There were no significant differences in perfusate gases, pH, and base excess (comparing both arterial and coronary venous samples) in these different groups of hearts (Table 4).

**DISCUSSION**

In an earlier experiment, the influences of pretreatment with acetaminophen on function and ultrastructure in the postischemia, reperfused myocardium was investigated (21). In that experiment, 0.35 mmol/l acetaminophen was administered 20 min before the onset of a 20-min period of global, low-flow myocardial ischemia followed by 40 min of reperfusion. Thus hearts in that study were exposed to acetaminophen for ~40 min before the onset of reperfusion and for 80 min by the end of the period of reflow. In the present study, the same concentration of acetaminophen was administered 10 min after the onset of ischemia. By the time of reperfusion, these hearts had been treated for only 10 min with acetaminophen and for only 50 min by the end of the period of reflow. Still, acetaminophen-treated hearts regained a significantly greater fraction of their baseline, control function than vehicle-treated hearts during the period of reflow, and the myofibrillar ultrastructure was preserved. This comparison reveals that acetaminophen is efficacious during reperfusion.

**Ischemia-Reperfusion, Protein Oxidation, and Acetaminophen**

Vehicle-treated hearts revealed a significantly greater ($P < 0.05$) content of protein carbonyls during ischemia than was seen in the presence of acetaminophen (Table 5). However, in both groups, protein carbonyls were still significantly elevated above corresponding baseline, control values after 40 min of reperfusion.
even when administered midway through a brief period of ischemia. Whether or not hearts would be protected by administration of acetaminophen upon initiation of reperfusion remains to be seen.

Any functional and/or structural differences between vehicle- and acetaminophen-treated hearts in the current study cannot be explained on the basis of changes over time. In our timed, control protocol, we found that two groups of hearts receiving no treatments other than an initial administration of acetaminophen (0.35 mmol/l) and vehicle could not be differentiated statistically during the 90-min experimental period. It was clear, however, that a trend toward improved function with time occurred in the acetaminophen-treated hearts, i.e., all indexes of left ventricular mechanical function appeared to improve with time. Thus comparing the current results with those from our previous study suggests that the longer the myocardium is exposed to acetaminophen, the more efficacious it becomes. This implies that hearts treated chronically

Table 3. Influence of urate in the absence and presence of acetaminophen on peroxynitrite-mediated chemiluminescence

<table>
<thead>
<tr>
<th></th>
<th>Integrated Counts Per Minute, $\times 10^{-7}$</th>
<th>Time to Peak Counts Per Minute, min</th>
<th>Half-Time of Peak Counts Per Minute, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>3.8 ± 1.2</td>
<td>14 ± 2</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>0.4 ± 0.05*</td>
<td>18 ± 2*</td>
<td>21 ± 2*</td>
</tr>
<tr>
<td>Urate</td>
<td>0.6 ± 0.1*</td>
<td>19 ± 1</td>
<td>22 ± 3*</td>
</tr>
<tr>
<td>Acetaminophen + urate</td>
<td>0.4 ± 0.04*</td>
<td>18 ± 3*</td>
<td>20 ± 1*</td>
</tr>
</tbody>
</table>

Data are means ± SE; $n = 5$ guinea pigs each. Half-time of peak counts per minute is the time required for peak counts per minute to diminish by 50%; *$P < 0.05$ relative to corresponding vehicle values. Note that acetaminophen, urate, and acetaminophen + urate values do not differ significantly from one another.
with acetaminophen might be expected to function better during ischemia-reperfusion than those treated with vehicle.

Hydrogen Peroxide and Acetaminophen

Exposure of the intact adult guinea pig myocardium to 0.1 mmol/l hydrogen peroxide for 15 min produced no significant, observable effects on the variables recorded in our current experiment. Conversely, as the concentration was increased to 0.25 and 0.4 mmol/l, there was considerable dysfunction, as evidenced by the marked reductions in ventricular contractile function. Although not shown here, several vehicle-treated hearts in preliminary experiments fibrillated after a few minutes of exposure to concentrations of 0.4–1.0 mmol/l hydrogen peroxide. These concentrations resulted in rapid and precipitous decrements in left ventricular function. In the same preliminary experiments, ventricular fibrillation was not seen when acetaminophen-treated hearts were exposed to similarly high concentrations of hydrogen peroxide.

The effects of hydrogen peroxide on the mammalian myocardium are influenced, among other things, by the tissue preparations and hydrogen peroxide concentrations being investigated (6, 14). There is some evidence of protein damage, and target proteins include extracellular signal-regulated kinases, mitogen-activated protein kinases, and native myoglobin, to name a few (3, 8). Many of the pathophysiological effects of hydrogen peroxide can be prevented by a 15-min pretreatment with 0.2 mmol/l salicylaldehyde isonicotinoyl hydrazine (SIH). This novel lipophilic iron chelator is known to attenuate the oxidative stress of hydrogen peroxide by reducing its ability to release hydroxyl radical via the Fenton reaction (2, 14).

We did not quantify the electrophysiological effects of hydrogen peroxide in the current study but did note its ability, in both groups, to produce occasional ventricular premature beats (VPB) and salvos (VS) (data not shown). It has been previously reported that acetaminophen can reduce the incidence of pentobarbital sodium-induced VPBs and VS (19), but the influence of acetaminophen on hydrogen peroxide-induced ventricular arrhythmias was not investigated nor was electronmicroscopy of the hydrogen peroxide-damaged myocardium done. However, acetaminophen is able to attenuate the production of hydroxyl radicals during reperfusion (21). Thus it seems reasonable to suggest that the protective effects of acetaminophen against hydrogen peroxide are mediated via a similar mechanism, i.e., by inhibition of the actions of hydroxyl radicals released from hydrogen peroxide in the Fenton reaction (2, 14).

Ischemia-Reperfusion, Peroxynitrite, and Acetaminophen

There is still general controversy over the potential toxic versus beneficial effects of peroxynitrite in the mammalian myocardium (11, 23). We chose to study peroxynitrite during ischemia-reperfusion injury because of its ability to be detected by chemiluminescence (30, 31), a relatively simple chemical assay. In our experiments, peroxynitrite was generated from the coproduction of nitric oxide and superoxide. These two precursors, in turn, were donated via a cascade of oxidation/reduction reactions beginning with exogenous linsidomine (SIN-1).

When 0.35 mmol/l acetaminophen was administered midway through a 20-min period of global, low-flow myocardial ischemia, hearts regained greater contractile function during the subsequent period of reperfu-
sion than vehicle-treated hearts. Coronary venous perfusate samples collected in vehicle-treated hearts displayed greater chemiluminescence during reperfusion than those collected from hearts treated with acetaminophen. These results are qualitatively similar to those obtained when the same concentration of acetaminophen was administered 20 min before the onset of low-flow ischemia. Moreover, when peroxynitrite was sequestered by urate, it was unable to oxidize luminol to produce the blue light of chemiluminescence. Thus urate had effects similar to those of acetaminophen. The mechanism of acetaminophen-mediated cardioprotection in the current study appears to be similar to that seen when acetaminophen was administered before the onset of ischemia (21), i.e., attenuation of the oxidation of luminol by peroxynitrite. Peroxynitrite is a potent oxidant, and, like hydrogen peroxide, hydroxyl radical, and other reactive oxygen species, peroxynitrite can alter the structure and function of nucleic acids, proteins, lipids, and other macromolecules (11, 17, 33, 35). Thus, by attenuating the damaging effects of powerful cytotoxic oxidants, acetaminophen is able to preserve the structural integrity and physiological function of the mammalian myocardium.

Ischemia-Reperfusion, Protein Oxidation, and Acetaminophen

Gow et al. (13) and Ischiropoulos and Al-Mehdi (15) have reported peroxynitrite-mediated oxidative injury to proteins. Powell and Tortolani (25) have reported the ability of antioxidants, including salicylate, to protect against such injury during ischemia and reperfusion in isolated rodent hearts. Our measurement of cytosolic protein carbonyls, another expression of tissue damage, gives additional information about the antioxidant, cytoprotective properties of acetaminophen. The content of protein carbonyls in the injured myocardium treated with acetaminophen was significantly less during ischemia than was seen in vehicle-treated hearts. However, after 40 min of reperfusion, protein carbonyls were still elevated in both groups. Thus whatever protective effect acetaminophen had during ischemia appeared to wane during reperfusion. More work is needed to explain this phenomenon and to begin identifying specific proteins that are spared during ischemia. From our electronmicrographs and the data of others (8, 23, 24), it seems apparent that both functional and structural proteins are involved.

Limitations

While isolated, perfused rodent hearts, such as the Langendorff preparation used in the current study, offer several advantages over other preparations (e.g., elimination of confounding neurogenic factors, ease of methodology, etc.), they do have significant limitations. We used a crystalloid perfusate as opposed to one with colloid (e.g., bovine serum albumin) and/or cellular elements. The potential problems associated with the disposition of peroxynitrite of differing perfusates are still under debate (18, 27). For example, red blood cells, in addition to improving the oxygen content of the perfusate, can release/take up glutathione, which in turn can modify the metabolism of peroxynitrite (32).

Summary and Conclusions

In the isolated, perfused guinea pig heart exposed to low-flow global myocardial ischemia and reperfusion, acetaminophen is cardioprotective. In a previous study, the efficacy when acetaminophen was administered ~20 min before the onset of ischemia was described (21). It is evident from the current study that acetaminophen is efficacious even when administered during ischemia. This significant difference advances our understanding of the time course of the cardioprotective actions of acetaminophen. It supports the need to continue the investigation by administering acetaminophen at the onset of reperfusion and to look at other time frames as well. Antioxidant mechanisms involving peroxynitrite, hydroxyl radical, and hydrogen peroxide appear to be involved. Additional studies are needed to determine the ability of acetaminophen to influence other perturbations, e.g., cytokine- and hypoxia/reoxygenation-mediated tissue injury.

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REFERENCES


