Acetaminophen in the Post-ischemia Reperfused Myocardium

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Acetaminophen was administered acutely at the onset of reperfusion after 20 min of low-flow, global myocardial ischemia in isolated, perfused guinea pig hearts (Langendorff) to evaluate its influence in the postischemia, reperfused myocardium. Similarly prepared hearts were treated with vehicle or with uric acid (another phenol for comparison). Functionally, acetaminophen-treated hearts (0.35 mM) achieved significantly greater recovery during reperfusion. For example, left ventricular developed pressures at 40 min reperfusion were 38 ± 3, 27 ± 3, and 20 ± 2 in the presence of acetaminophen ($P < 0.05$, relative to the other two groups), vehicle, and uric acid, respectively. Coronary perfusion pressures and calculated coronary vascular resistances, in the acetaminophen-treated hearts, were significantly lower at the same time (e.g., coronary perfusion pressures in the three groups, respectively, were 40 ± 2 [$P < 0.05$], 51 ± 3, and 65 ± 12 mm Hg). Under baseline, control conditions, creatine kinase ranged from 12–15 units/liter in the three groups. It increased to 35–40 units/liter ($P < 0.05$) during ischemia but was significantly reduced by acetaminophen during reperfusion (e.g., 5.3 ± 0.8 units/liter at 40 min). Oxidant-mediated chemiluminescence in all three treatment groups during baseline conditions and ischemia was similar (i.e., approximately 1.5–2.0 min for peak luminescence to reach its half maximal value). It took significantly more time during reperfusion for the oxidation of luminol in the presence of acetaminophen (>20 min, $P < 0.05$) than in its absence (3–8 min in uric acid- and vehicle-treated hearts). These results suggest that administration of acetaminophen (0.35 mM), at the onset of reperfusion, provides anti-oxidant–mediated cardioprotection in the postischemia, reperfused myocardium. Exp Biol Med 227:1031–1038, 2002

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It has been about 100 years since the introduction of acetaminophen (paracetamol, APAP) to human medicine (1–3). Although its pain-relieving and temperature-lowering actions have been under investigation for several decades, its actions on the cardiovascular system and other mammalian organ systems have not been elucidated. For example, acetaminophen's effects on vascular beds such as cerebral, splanchnic, renal, and skeletal muscle have yet to be published. Similarly, no laboratory has undertaken a systematic investigation of the potential positive versus negative inotropic/chronotropic/dromotropic properties of acetaminophen in the heart. Acetaminophen has been used in western medicine for many decades (1–3), and it seems unusual that such information has not been available previously.

Acetaminophen is a phenol (4), and many phenols have antioxidant properties (5, 6). Oxidative stress in tissues has been a topic of intense investigation both in the basic science laboratory and in clinical research in recent years (7, 8). Taking basic discoveries in oxidative stress to a clinical application, i.e., translational research, is of significant contemporary interest (9–11). Increased use of acetaminophen by an aging population (12) justifies a more indepth exploration into the unknown mechanisms of action of this compound. Such has been an important objective of our recent investigations (13–15). In one of these studies, we found evidence of antiarrhythmic properties of acetaminophen (14). In a preliminary experiment (unpublished results), we found concentration-dependent positive inotropic actions of acetaminophen and modest, dose-dependent, coronary vasoconstriction. In the current article we report significant, anti-oxidant–mediated, cardioprotective actions of acetaminophen when it is delivered, upon reperfusion, to the postischemia myocardium.

Materials and Methods

Animals and Heart Preparation. Following NIH/USDA 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 7–14 days to acclimate to 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.* (16, 17) and modified by Wei *et al.* (18). Instrumentation included inserting a flaccid latex balloon into the left atrium and advancing it across the mitral valve into the left ventricle, where it was filled with Krebs-Henseleit buffer solution (KHB) to an end–diastolic pressure of 0–5 mm Hg (volume of approximately 75–100 μl). A pacing electrode was attached to the base of the right ventricle, and a large bore polyethylene cannula (PE240) 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 Inc., Clifton, NJ). All hearts in all protocols were instrumented the same and were perfused retrogradely via the cannulated aorta without recirculation.

Upon completion of instrumentation, hearts were perfused in increments of approximately 2 ml/min at 3- to 4-min intervals until a control rate of 7 ml/min was reached. Flow was controlled at this rate throughout the experiment except during low-flow ischemia, when it was reduced to about 1 ml/min (Peri-Star pump, model 291, World Precision Instruments, Sarasota, FL). Hearts were allowed approximately 30 min post-instrumentation for monitored variables to achieve steady-state conditions. Monitored variables included: heart rate (HR, paced at spontaneous rate plus approximately 15%, cpm [cycles per minute], left ventricular developed pressure (LVDP, mm Hg; the difference between peak systolic and end–diastolic pressures in the left ventricle), $\pm dP/dt_{max}$ (mm Hg/sec, contractility), pressure rate product (PRP, LVDP × HR, mm Hg/cpm), coronary perfusate flowrate (CPF, ml/min), coronary perfusion pressure (CPP, mm Hg), and calculated coronary vascular resistance (CVR, mm Hg/ml/min).

Perfusate and Perfusion Modality. Perfusate was a modified Krebs-Henseleit physiological buffer solution (vehicle for acetaminophen) containing (in mM): NaCl (128.0), KCl (4.7), $MgSO_4 \cdot 7H_20$ (1.5), $CaCl_2$ (2.5), KH_2PO_4 (1.2), $NaHCO_3$ (24.9), glucose (10.0), pyruvate (2.0), and insulin (200 μU/ml). It was warmed to 38°C and equilibrated with 95% O_2 and 5% CO_2 (pH 7.40 ± 0.02). Aortic flow (antegrade coronary flow) was achieved retrogradely from a water-jacketed 1.5-liter reservoir, and was continuously monitored ultrasonically (model 2N423 flowprobe, model T101 flowmeter, Transonic Inc., Ithaca, NY).

LVDP was monitored isovolumetrically (model P231D, Gould-Statham, Oxnord, CA) and inflow (coronary arterial) and outflow (pulmonary arterial, i.e., coronary venous) perfusate samples were collected anaerobically for monitoring pH and perfusate gases (0.5–1.0 ml). Standard electrodes were used to measure partial pressures of oxygen (PO_2, mm Hg), and carbon dioxide (PCO_2, mm Hg), as well as pH, CO_2 content, and base excess (model 248 blood gases/pH analyzer, Bayer Diagnostics, Norwood, MA) as previously reported from this (18–20) and other laboratories (21).

Experimental Protocols. Four experimental protocols (i–iv) were conducted (see details below). (i) This experiment was conducted to determine the efficacy of acetaminophen (against dysfunction/injury during reperfusion) when administered at the onset of reperfusion, postischemia. (ii) The second experiment was conducted to compare and contrast the efficacy of uric acid (2,6,8-trihydroxypurine, another phenol) with acetaminophen. This agent was also administered at the onset of reperfusion. (iii) A third experiment compared the effects of vehicle, acetaminophen, and uric acid on the production of blue light (chemiluminescence) during reperfusion (agents administered at the onset of reperfusion to identify their antioxidant/anti-chemiluminescent actions). (iv) Finally, in a fourth experiment, we measured the production of creatine kinase, a biochemical marker of tissue damage in myocardial ischemia and reperfusion. In this experiment efficacies of all three agents were compared.

Actions of Acetaminophen When Administered at Onset of Reperfusion. The objective of this experiment was to determine whether administering acetaminophen at the onset of reperfusion, after a 20-min period of low-flow, global myocardial ischemia, would be efficacious in the injured, postischemia reperfused myocardium. The endpoints for determining efficacy were left ventricular and coronary circulatory functions. After each heart was instrumented and when monitored variables were in the steady state, a set of baseline (control) data were collected. Subsequently, hearts were submerged in warmed KHB (to maintain temperature during ischemia) and CPF was reduced to approximately 1 ml/min for 20 min. We have previously reported no aphysiological effects of submerging Langendorff-perfused guinea pig hearts in physiological salt solutions (20). As long as the hearts are adequately oxygenated, they continue to beat rhythmically and contract physiologically. Data, including samples of venous effluent perfusate, were collected only at 10 and 20 min of ischemia. Shortly before the period of ischemia either KHB or acetaminophen (0.35 mM, final concentration in the perfusate reservoir) was added to the perfusate reservoir. Timing and the dead space volume in the perfusion system were accurately calculated so the agent would begin arriving at the heart at the onset of reperfusion. After 20 min of ischemia, the heart chamber was drained (i.e., hearts were removed from submersion fluid) and CPF was restored to its preischemia level. Monitored variables (e.g., developed pressure, coronary perfusion pressure), including samples of ve-

nous effluent, were collected at 1, 3, 6, 10, and 40 min of reperfusion.

Uric Acid and the Reperfused Myocardium. Our main objective in this experiment was to compare another phenol, uric acid, with acetaminophen in the injured, reperfused myocardium. Uric acid is a circulating anti-oxidant in human plasma and has been of recent interest to one of the authors (KVD). This experiment was designed identically to that described above. The only difference was the replacement of acetaminophen with uric acid. In preliminary experiments we completed concentration/response studies to help identify an effective concentration of uric acid, i.e., one that could attenuate the production of peroxynitrite but that was not immediately toxic to the heart. We evaluated concentrations ranging from 0.1–0.4 mM. We used 0.18 mM because it was in the middle of this range and because it had no direct effects on the heart under baseline conditions. Data were collected at each of the eight time intervals described above.

Peroxynitrite and Acetaminophen Versus Uric Acid. Three groups of hearts were studied; vehicle treated (n = 6), acetaminophen treated (n = 6), and uric acid treated (n = 6). Samples of coronary venous effluent perfusate (0.25–0.5 ml each) were collected in prechilled 1.5-ml capped vials at baseline, 10, and 20 min ischemia, and 1, 3, 6, 10, and 40 min reperfusion, i.e., eight samples from each heart. Samples were stored at −70°C until analysis. Chemiluminescence technology was used to assess the production of peroxynitrite as previously described (5, 6, 13).

Creatine Kinase in Vehicle-, Acetaminophen-, and Uric Acid-Treated Hearts. Creatine kinase (CK) is a well-established marker of myocardial injury during ischemia and reperfusion (22–24). In this experiment we compared the effects of all three agents on the release of creatine kinase (n = 3–6 hearts per treatment group). Standard assays were used (product no. 47-10, Sigma Diagnostics, St. Louis, MO) and were based on the modified procedures of Nielsen and Ludvigsen (25) and Rosalki (26). Briefly, 1 ml of reconstituted reagent was added to 20 μl of sample in a 1 ml minimum cuvette. Each sample was incubated for 3 min at 30°C (±2°C) and processed spectrophotometrically at 340 nm (30-sec intervals for a total of 120 sec). Data are expressed in units per liter, where 1 unit of activity is defined as the amount of enzyme that produces 1.0 μmol/min of NADH.

Statistical Analysis. All data are presented as means plus or minus one SEM. Differences in group variances were identified using analysis of variance (ANOVA, repeated measures design). Differences in group means were identified using *a priori* tests including Fisher's (least significant difference) and Tukey's w-procedure. Significance was established at $P < 0.05$ in all cases.

Results

Ventricular and Coronary Vascular Function. Under baseline conditions LVDP was approximately 35–40 mm Hg in all hearts in each of the three groups. During ischemia, this decreased to about 5–10 mm Hg at 10 min and remained at these levels throughout ischemia. In the first 3 min of reperfusion, there were no obvious differences in LVDP among the three treatment groups. By 6 min of reperfusion, uric acid-treated hearts were developing significantly lower ventricular pressures than hearts in the other two groups. This trend persisted through the remaining 30 plus minutes of reperfusion and was indicative of other indices of left ventricular mechanics (e.g., PRP, $\pm dP/dt_{max}$). Although LVDP values in acetaminophen-treated hearts were consistently higher than those in vehicle-treated hearts, these differences did not achieve statistical significance until 40 min of reperfusion (Fig. 1)

Coronary perfusion pressure was 38 ± 2 mm Hg under baseline, pre-ischemia control conditions in hearts in all three groups. Coronary perfusion pressure dropped to approximately 10–15 mm Hg during ischemia and did not differ significantly amongst the various treatment groups. Upon reperfusion, CPP in vehicle-treated hearts increased slowly but steadily, becoming significantly greater than that in acetaminophen-treated hearts by 40 min of reperfusion. Coronary perfusion pressure in acetaminophen-treated hearts returned to baseline levels in the first minute of reperfusion, and maintained this plateau throughout reperfusion (Fig. 2). Treatment with uric acid caused marked, statistically significant increments in CPP throughout the period of reperfusion. Changes in calculated CVR in the three groups paralleled changes in CPP and are summarized, with other indices of mechanical function, in Tables I and II.

Effects of Acetaminophen, Uric Acid, and Vehicle on Peroxynitrite. The peroxynitrite-mediated oxidation of luminol and accompanying production of blue light (chemiluminescence) was evident in vehicle-treated hearts as early as 1 min of reperfusion (Fig. 3). This initial burst, and the subsequent production of blue light, was abol-

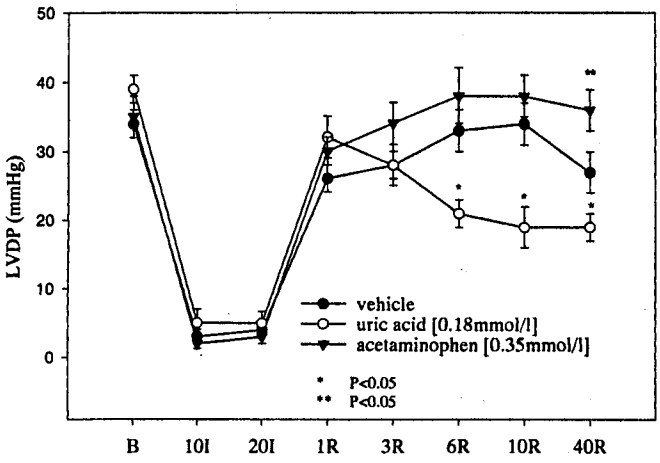

Figure 1. Influence of ischemia/reperfusion on LVDP in the presence of vehicle, uric acid, and acetaminophen. All three agents were administered at the onset of reperfusion. *$P < 0.05$ relative to other treatment groups; **$P < 0.05$ relative to vehicle. B, baseline, control conditions; I10, 10 min of ischemia; I20, 20 min of ischemia; R1–R40, 1, 3, 6, 10, and 40 min of reperfusion.

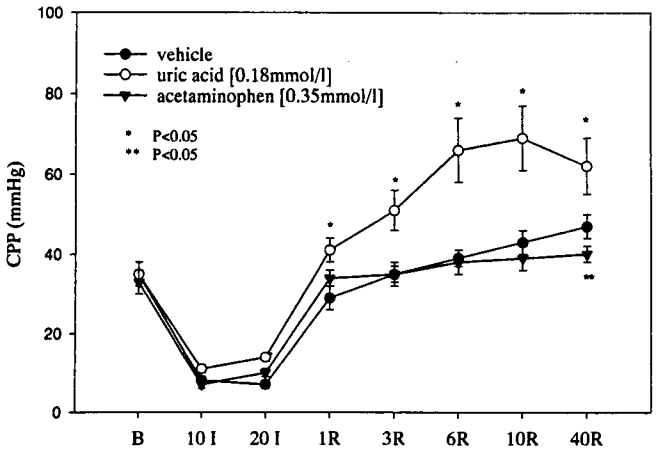

Figure 2. Influence of ischemia/reperfusion on CPP in the presence of vehicle, uric acid, and acetaminophen. *$P < 0.05$ relative to the other treatment groups; **$P < 0.05$ relative to vehicle. B, baseline, control conditions; I10/I20, 10 and 20 min of ischemia; R1–R40, 1, 3, 6, 10, and 40 min of reperfusion. Note the stability of coronary perfusion pressure during reperfusion in the acetaminophen-treated group.

ished during the 40 min of reperfusion in the presence of acetaminophen. During the first 3 min of reperfusion, uric acid also attenuated the production of blue light (when compared with vehicle-treated hearts). However, at 6–10 min, there were no significant differences between vehicle- and uric acid-treated hearts, and by 40 min chemiluminescence in the presence of uric acid was elevated significantly above that for vehicle.

The kinetics of peroxynitrite-mediated chemiluminescence are summarized in Table III. For example, the t½ (min) for both the production and abatement of blue light, in the presence of acetaminophen, exceeded the predetermined detection time of the luminometer (20 min).

Effects of Acetaminophen, Uric Acid, and Vehicle on Creatine Kinase. Under baseline, control conditions, the pooled data revealed creatine kinase concentrations in the range of 12–15 units/liter. These were increased significantly by ischemia to approximately 35–40 units/liter in all three groups. Relative to ischemia, washout of creatine kinase in all three treatment groups was evident in the early minutes of reperfusion. For example, in acetaminophen-, uric acid-, and vehicle-treated hearts, CK values increased from baseline levels to 36 ± 14 ($P < 0.05$), 35 ± 8 ($P < 0.05$), and 36 ± 6 ($P < 0.05$), respectively. At 1 min reperfusion, corresponding CK values in these three treatment groups ranged from 5–10 units/liter. During 3 and 6 min of reperfusion, CK levels remained near baseline/ischemia levels in vehicle- and uric acid-treated groups. Conversely, CK concentrations in acetaminophen-treated hearts were significantly reduced ($P < 0.05$) at 3, 10, and 40 min reperfusion relative to vehicle- and uric acid-treatment groups. In fact, when compared with its own baseline, control value, CK was significantly reduced by acetaminophen during much of the period of reperfusion (Fig. 4).

Discussion

Recovery of Function. If myocardial function is expressed as left ventricular developed pressure and coronary perfusion pressure/calculated coronary vascular resistance, then acetaminophen-treated hearts were more functional by the end of reperfusion than hearts treated either with uric acid or vehicle. This trend was observable as early as 1–6 min of reperfusion when comparing vehicle with acetaminophen. When comparing acetaminophen with uric acid the results for developed pressure and coronary perfusion pressure were even more pronounced. The differences in the three treatment groups is most likely explained on the basis of the more effective removal of damaging oxygen radicals by acetaminophen throughout the period of reperfusion. This explanation is consistent with earlier results obtained in this laboratory with reperfusion and damaging oxidants (13, 27), and suggests salutary effects of acetaminophen on both the injured coronary vasculature and on the myocardial parenchyma.

Uric acid is of physiologic importance to humans because it is the terminal oxidation product of purine metabo-

Table I. Influence of Ischemia and Reperfusion on Hemodynamic Variables in the Presence of Vehicle (V), Acetaminophen (A), and Uric Acid (U) in Isolated, Perfused Hearts

	CPF (ml/min)			CVR (mmHg/ml/min)			HR (cycles/min)		
	V	A	U	V	A	U	V	A	U
Basal	7.0 ± 0.5	7.0 ± 0.5	7.0 ± 0.5	5.0 ± 0.1	4.8 ± 0.1	5.0 ± 0.2	270 ± 20	270 ± 20	270 ± 20
Ischemia									
10 min	1.0 ± .05	1.0 ± .05	1.0 ± .05	7.2 ± 0.4	7.3 ± 0.4	8.3 ± 0.5	270 ± 20	270 ± 20	270 ± 20
20 min	1.0 ± .05	1.0 ± .05	1.0 ± .05	7.1 ± 0.3	7.6 ± 0.7	8.1 ± 0.7	270 ± 20	270 ± 20	270 ± 20
Reperfusion									
1 min	7.0 ± 0.5	7.0 ± 0.5	7.0 ± 0.5	4.2 ± 0.1	5.3 ± 0.2	5.8 ± 0.2	270 ± 20	270 ± 20	270 ± 20
3 min	7.0 ± 0.5	7.0 ± 0.5	7.0 ± 0.5	4.6 ± 0.2	5.0 ± 0.1	7.3 ± 0.3*	270 ± 20	270 ± 20	270 ± 20
6 min	7.0 ± 0.5	7.0 ± 0.5	7.0 ± 0.5	5.6 ± 0.2	5.5 ± 0.1	9.4 ± 0.6*	270 ± 20	270 ± 20	270 ± 20
10 min	7.0 ± 0.5	7.0 ± 0.5	7.0 ± 0.5	6.1 ± 0.4	5.4 ± 0.3	9.8 ± 0.8*	270 ± 20	270 ± 20	270 ± 20
40 min	7.0 ± 0.5	7.0 ± 0.5	7.0 ± 0.5	6.7 ± 0.5	5.6 ± 0.4**	8.9 ± 0.7*	270 ± 20	270 ± 20	270 ± 20

Data are means ± 1 s.e.m. (n = 8–12 per group). CPF, coronary perfusate flowrate (controlled); CVR, calculated coronary vascular resistance (see Fig. 2 for corresponding coronary perfusion pressures); HR, heart rate (paced at spontaneous rates plus about 15 percent); *, $P < 0.05$ relative to corresponding values in vehicle and acetaminophen columns; **, $P < 0.05$ relative to corresponding value in vehicle column.

Table II. Influence of Ischemia and Reperfusion on Ventricular Mechanics in the Presence of Vehicle (V), Acetaminophen (A), and Uric Acid (U) in Isolated, Perfused Hearts

	PRP (mmHg x cpm)			$+dP/dt_{max}$ (mmHg/sec)			$-dP/dt_{max}$ (mmHg/sec)		
	V	A	U	V	A	U	V	A	U
Basal	9674 ± 692	10097 ± 495	10093 ± 560	284 ± 13	279 ± 15	281 ± 17	241 ± 18	232 ± 18	240 ± 15
Ischemia									
10 min	692 ± 256	510 ± 239	554 ± 224	32 ± 7	31 ± 7	28 ± 8	17 ± 3	17 ± 3	18 ± 5
20 min	1006 ± 328	980 ± 409	838 ± 464	36 ± 8	41 ± 7	41 ± 9	20 ± 4	22 ± 2	21 ± 6
Reperfusion									
1 min	7255 ± 1088	8295 ± 1076	7821 ± 823	239 ± 17	258 ± 17	239 ± 25	169 ± 14	185 ± 10	177 ± 16
3 min	7973 ± 1188	10175 ± 1072	7317 ± 718	258 ± 16	294 ± 23	239 ± 21	204 ± 8	223 ± 13	177 ± 16
6 min	8521 ± 537	9934 ± 506	5538 ± 552†	250 ± 13	276 ± 17	160 ± 22†	204 ± 12	220 ± 11	112 ± 16†
10 min	8780 ± 534	9621 ± 550	4804 ± 540†	252 ± 12	263 ± 22	136 ± 18†	188 ± 15	217 ± 15	89 ± 12†
40 min	7413 ± 446	8846 ± 509*	4398 ± 496†	223 ± 14	252 ± 12*	126 ± 12†	166 ± 11	197 ± 19*	87 ± 11†

Data are means ± 1 s.e.m. (n = 8–12 per group). PRP, product of left ventricular developed pressure and heart rate; $+dP/dt_{max}$, first derivative of left ventricular pressure development (i.e. contractility); $-dP/dt_{max}$, second derivative of left ventricular pressure (i.e. rate of ventricular relaxation); †, $P < 0.05$ relative to corresponding values in columns marked V and A; *, $P < 0.05$ relative to corresponding values in column marked V.

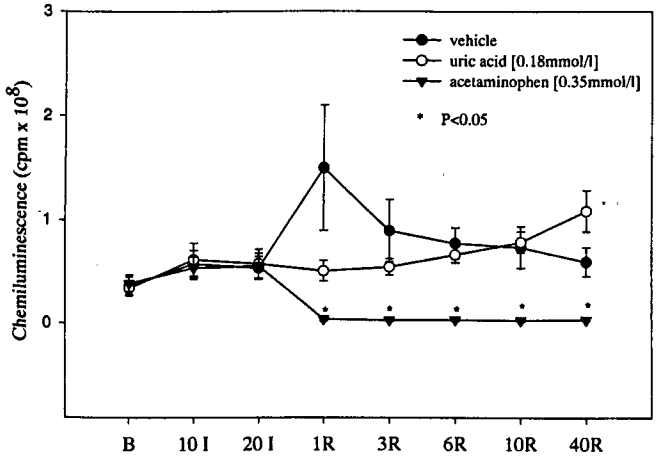

Figure 3. Influence of ischemia/reperfusion on peroxynitrite-mediated, luminol-dependent, chemiluminescence in the presence of vehicle, uric acid, and acetaminophen. *$P < 0.05$ relative to other treatment groups. B, baseline, control conditions; I10/I20, 10 and 20 min of ischemia; R1–R40, 1, 3, 6, 10, and 40 min reperfusion. Note the complete absence of chemiluminescence in the presence of acetaminophen, and the progressive elevation after 6 min in the presence of uric acid.

lism (28). Human plasma contains uric acid at concentrations approaching 0.5 mM (29), it is a polyphenol, and such antioxidants are the circulatory system's first line of defense against oxidant-induced tissue injury (11). Our initial objective was to compare equimolar concentrations of uric acid and acetaminophen. However, at concentrations approaching 0.35 mM marked cardiotoxicity (e.g., concentration-dependent negative inotropy) was evident with uric acid. We are well aware that by the laws of mass action, we supplied only about half the antioxidant concentration of uric acid as acetaminophen. Thus, comparing the two under the present circumstances might not be justified. However, as can be seen from the data, e.g., Figs. 1, 2, and 4, even 0.18 mM uric acid seemed to be cardiotoxic during our ischemia/reperfusion protocol. In the presence of peroxynitrite, uric acid is nitrated to an as-yet-unidentified uric acid derivative (30). This product might play a pivotal role in tissue pathophysiology, producing cardiovascular as well as non-cardiovascular actions. Recent epidemiologic studies reveal that elevated uric acid concentrations are an independent risk factor for cardiovascular mortality in the general human population (31).

The maintenance of coronary perfusion pressure and coronary vascular resistance by acetaminophen during reperfusion (relative to vehicle and uric acid) is also consistent with its antioxidant actions as seen in this and our earlier studies (13–15). Nor is the coronary perfusion pressure of approximately 40 mm Hg under baseline conditions aphysiological for this guinea pig heart preparation. For example, at a coronary perfusion pressure of 40 mm Hg and a controlled flow rate of 7 ml/min, these hearts are neither ischemic nor hypoxic. They have coronary flow reserves of at least 100% (in some cases it is 2- to 3-fold greater than baseline values), and normal oxygen delivery, extraction, and consumption for crystalloid-perfused Langendorff hearts (personal observations).

Peroxynitrite. The burst of blue light seen in the early minutes of reperfusion in the presence of vehicle is consistent with our earlier observations. We have attributed the oxidation of luminol to the actions of peroxynitrite. However, ischemia/reperfusion alters the metabolism of purines in the myocardium and increases the activity of enzymes such as xanthine oxidase and nitric oxide synthase. Byproducts of this activity, e.g., superoxide and nitric oxide, are also known to oxidize luminol (5, 6, 32). Clearly, there are other nonidentified myocardial oxidants that can cause the production of luminol-dependent blue light. Thus, at the present, we are only able to conclude that peroxynitrite, generated by the combination of superoxide and nitric oxide, is possibly one of several potential targets of the antioxidant actions of acetaminophen. Still, in the presence of acetaminophen chemiluminescence was markedly attenuated (e.g., see Table III). The increased maximum slope, t½ rise, and t½ decline denote marked attenuation of the ability of peroxynitrite (other oxidants) to oxidize luminol to produce blue light. This is consistent with important cardioprotective properties of acetaminophen.

Table III. Influence of Vehicle (V), Acetaminophen (A), and Uric Acid (U) on Peroxynitrite-Mediated Chemiluminescence in the Post-Ischemia, Reperfused Myocardium

	TIME (min)								
	Maximum slope			t½ rise			t½ decline		
	V	A	U	V	A	U	V	A	U
Basal	3.2 ± 0.4	2.9 ± 0.5	3.1 ± 0.3	1.8 ± 0.2	1.6 ± 0.2	1.6 ± 0.3	5.2 ± 0.8	5.0 ± 0.7	5.1 ± 0.9
Ischemia									
10 min	3.8 ± 0.6	3.9 ± 0.4	3.7 ± 0.4	2.1 ± 0.3	2.1 ± 0.2	1.9 ± 0.1	6.3 ± 0.7	6.2 ± 0.5	5.9 ± 0.6
20 min	4.0 ± 0.6	4.2 ± 0.8	3.9 ± 0.6	2.0 ± 0.4	1.9 ± 0.1	1.8 ± 0.3	6.0 ± 0.5	5.9 ± 0.6	5.8 ± 0.5
Reperfusion									
1 min	6.2 ± 0.3	>20*	4.8 ± 1.2**	3.2 ± 0.4	>20*	2.8 ± 0.1**	7.6 ± 0.8	>20*	5.6 ± 0.6**
3 min	6.5 ± 0.6	>20*	4.8 ± 1.4**	3.6 ± 0.4	>20*	2.7 ± 0.3**	7.8 ± 0.7	>20*	5.8 ± 0.4**
6 min	8.8 ± 1.2	>20*	6.2 ± 1.2**	4.8 ± 0.8	>20*	3.2 ± 0.5**	8.3 ± 0.9	>20*	6.2 ± 0.6**
10 min	9.4 ± 1.6	>20*	8.9 ± 2.1	5.2 ± 0.8	>20*	4.9 ± 0.9	9.6 ± 1.3	>20*	6.8 ± 0.6
40 min	12.3 ± 1.8	>20*	16.3 ± 3.6	8.6 ± 1.0	>20*	7.6 ± 1.6	11.8 ± 2.0	>20*	8.3 ± 0.4

Data are means ± 1 s.e.m. (n = 5–6 per group). Maximum slope, time (min) for the peroxynitrite-mediated production of blue light (chemiluminescence) to achieve its maximum rate; t½ rise, time (min) for the production of light to reach 50 percent of its maximum value; t½ decline, time (min) for the production of blue light to decline to 50 percent of its maximum value; *, $P < 0.05$ relative to corresponding V and U; **, $P < 0.05$ relative to corresponding V.

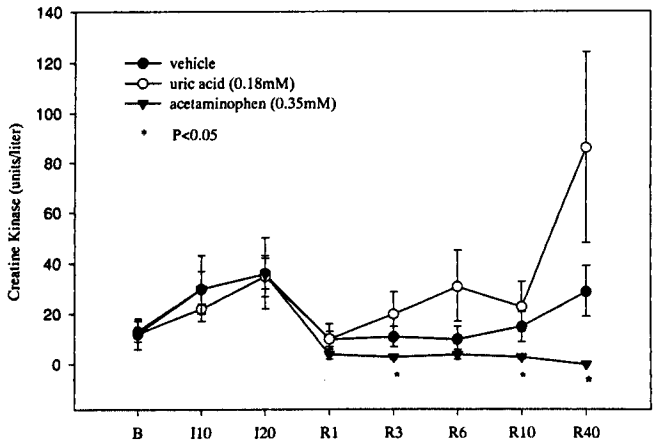

Figure 4. Influence of ischemia/reperfusion on creatine kinase in the presence of vehicle, uric acid, and acetaminophen. Note the tendency towards an increase during ischemia in all three groups, a further increase in the early minutes of reperfusion with uric acid and the reduction in variability during reperfusion with acetaminophen. *$P < 0.05$ relative to corresponding values in other groups at the same time.

Creatine Kinase. The concentrations of this important biochemical indicator of myocardial tissue damage varied within treatment groups, especially during ischemia and reperfusion. The most consistent result, however, was the marked, and statistically significant reduction in CK in the presence of acetaminophen during reperfusion. The expected effects of ischemia on CK were also observed, i.e., a significant increment during the 20-min period of restricted coronary perfusate flow (22). Importantly, CK concentrations did not vary significantly among the three treatment groups under baseline, control conditions, or during ischemia.

Others have reported changes in CK in isolated perfused hearts during ischemia/reperfusion (33–35), but there are no previous reports of the actions of acetaminophen on these changes. The significant reduction in CK in the presence of vehicle and acetaminophen in the early minutes of reperfusion are explained, in part, by washout. However, this trend was sustained in acetaminophen-treated hearts but not in the presence of either vehicle or uric acid. By the end of the period of reflow CK had returned to or above levels seen during baseline and ischemia conditions in the latter groups. Hence, washout cannot explain acetaminophen's actions in the later minutes of reperfusion. For example, by 10 and 40 min CK levels in the acetaminophen-treated group were lower than their own baseline/ischemia values. Clearly, the comparative results with uric acid and vehicle strengthen the argument that acetaminophen's cardioprotective properties are revealed at the biochemical, as well as the morphological and physiological (functional) levels (13, 14).

Time–Course and Therapeutic Doses of Acetaminophen. Our current interest in acetaminophen began a few years ago when we administered it to hearts 20 min before the onset of ischemia and found it to be beneficial (13). Later, we administered acetaminophen midway through a 20-min period of ischemia and also demonstrated cardioprotection (15). In this experiment, acetaminophen was introduced to the hearts at the onset of reperfusion. Collectively, these three experiments reveal that acetaminophen has important cardioprotective actions under all three temporal conditions. From our perspective, acetaminophen appears to be most efficacious when administered before the onset of ischemia. Time and additional experiments by others are needed to confirm this opinion. Nonetheless, our combined results raise questions about the potential beneficial effects of administering acetaminophen chronically (12).

The concentration of acetaminophen used in these experiments [0.35 mM] yields circulating concentrations in the coronary venous effluent perfusate of approximately

50 μg/ml. Several hours after therapeutic dosing, plasma concentrations in humans are typically 10–20 μg/ml, although values as high as 50 μg/ml have been reported. Cytotoxicity is generally thought to occur at plasma concentrations ≥300 μg/ml. Thus, 0.35 mM acetaminophen is greater than concentrations needed for therapeutic efficacy but is well below concentrations needed to produce hepato-/cytotoxicity. We have not conducted experiments using concentrations that would closely estimate therapeutic doses (e.g., 0.1–0.15 mM).

Summary and Conclusions. Administering acetaminophen at the onset of reperfusion following a 20-min period of low-flow, global myocardial ischemia, provides cardioprotection during reperfusion. Biochemical and functional evidence to support this conclusion have been presented here. Uric acid, an alternative polyphenol, provides some degree of cardioprotection (biochemical) in the early minutes of reperfusion, but is markedly cardiotoxic in the later minutes of reperfusion. This is true of concentrations that, under basal physiological conditions, show little or no signs of toxicity in our Landendorff heart preparation. There is mounting evidence that acetaminophen has important cardiovascular actions other than those mediated directly on the myocardium (36, 37). Clearly, more work on the cytoprotective properties of acetaminophen in the mammalian myocardium and other mammalian organ systems is warranted.

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