Attenuation of dysfunction in the postischemic 'stunned' myocardium by dimethylthiourea

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ABSTRACT The mechanism for the prolonged contractile dysfunction observed in myocardium reperfused after reversible regional ischemia ('stunned' myocardium) is unclear. Recent studies suggest that myocardial stunning may be mediated by oxygen-derived free radicals, but the precise molecular species involved remain unknown. Thus we explored the role of the highly cytotoxic hydroxyl radical in regional postischemic dysfunction by using dimethylthiourea (DMTU), an effective and highly permeable hydroxyl radical scavenger. Open-chest dogs undergoing a 15 min occlusion of the left anterior descending coronary artery followed by 4 hr of reperfusion received either DMTU (0.5 g/kg iv over 45 min starting 30 min before occlusion, n = 14) or saline (n = 15). Control and treated dogs were comparable with respect to variables that may affect postischemic dysfunction, including heart rate, aortic pressure, left atrial pressure, arterial blood gases and hemoglobin concentration, size of the occluded bed (determined by postmortem perfusion), and collateral blood flow (determined by radioactive microspheres). Regional myocardial function was assessed by measuring wall thickening with an epicardial Doppler probe. The two groups exhibited comparable systolic thickening under baseline conditions and similar degrees of dyskinesis during ischemia. After reperfusion, however, wall thickening (expressed as percent of baseline) was considerably greater in treated as compared with control dogs: 53 ± 9% (mean ± SEM) vs 9 ± 14% (p < .03) at 1 hr, 55 ± 9% vs 23 ± 13% (p < .05) at 2 hr, 60 ± 9% vs 28 ± 14% (p < .05) at 3 hr, and 67 ± 5% vs 36 ± 13% (p < .05) at 4 hr. Thus DMTU produced a significant and sustained improvement in recovery of contractile function. In concentrations greater than the plasma levels attained in vivo, DMTU did not scavenge either hydrogen peroxide or superoxide anion in vitro. These results suggest that the myocardial dysfunction occurring after a brief episode of regional ischemia is mediated in part by the hydroxyl radical.


MYOCARDIUM reperfused after a reversible ischemic insult exhibits prolonged contractile dysfunction ('stunned' myocardium).1-3 Despite considerable investigative efforts, the mechanism responsible for this phenomenon remains unclear. Recently, several studies have demonstrated that the recovery of the stunned myocardium is enhanced by agents that decrease the concentration of superoxide anion (·O₂⁻) and hydrogen peroxide (H₂O₂), such as superoxide dismutase plus catalase,1-6 N-2-mercaptopropionylglycine,7 and allopurinol,8 suggesting that accumulation of ·O₂⁻ and/or H₂O₂ plays an important role in the genesis of postischemic dysfunction.

These studies did not attempt to discern the relative roles of the various oxygen species in myocardial stunning. Both ·O₂⁻ and H₂O₂ are theoretically capable of producing myocellular damage in themselves. In addition, ·O₂⁻ and H₂O₂ can react to generate the hydroxyl radical (·OH), an oxidant considerably more potent than either of its precursors.9,10 Consequently, accumulation of ·O₂⁻ or H₂O₂ could produce postischemic dysfunction either directly, via the cytotoxic actions of these species, or indirectly, via ·OH generation. Administration of an ·OH scavenger may help discern between these two mechanisms because it would not interfere with injury caused directly by ·O₂⁻ or H₂O₂ but it should attenuate the toxic actions of ·OH.
·OH. To date, no data are available regarding the potential contribution of ·OH to regional myocardial stunning in the intact animal. Such information would not only help to elucidate the mechanisms of radical-mediated damage but may also have therapeutic implications.

Evaluation of the role of ·OH in tissue injury has been hindered by the fact that there are no known enzymatic scavengers of this radical and the chemical scavengers available (e.g., dimethyl sulfoxide, mannitol, ethanol) may lack potency or specificity. Recently, Fox et al. have described the ·OH-scavenging properties of dimethylthiourea (DMTU), a compound that is more effective than traditional ·OH scavengers and is highly cell permeable. We therefore used DMTU as a probe to explore the role of the ·OH radical in postischemic dysfunction. We employed a previously described open-chest canine preparation in which severe myocardial stunning is produced by a 15 min coronary occlusion followed by reperfusion. This duration of ischemia was selected because it is well established that it does not result in myocardial necrosis in the dog, although it does produce prolonged depression of contractility. In addition, studies in vitro were performed to (1) elucidate the specificity of DMTU for ·OH, (2) correlate the plasma DMTU levels attained in vivo with the effective ·OH-scavenging concentrations in vitro, and (3) determine the pharmacokinetics of DMTU.

Methods

This study was performed in accordance with the guidelines of the Committee on Animals of Baylor College of Medicine and with the "Guiding Principles in the Use and Care of Animals" approved by the American Physiological Society.

Measurement of oxygen metabolites in vitro. Previous studies have shown that DMTU is an effective scavenger of ·OH. We carried out additional experiments in vitro at the Medical College of Virginia to determine the specificity of DMTU; to this end, the ability of various concentrations of DMTU to scavenge ·O₂ and H₂O₂ was evaluated.

Superoxide generated as a result of xanthine oxidase (0.02 U/ml) action on xanthine (53 μM) was monitored by reduction of ferricytochrome c (22 μM, Type VI, Sigma Chemical Co.) at a wavelength of 550 nm in the presence or absence of superoxide dismutase (10 μg/ml). DMTU (1 to 10 mM) was included in the reaction mixture to determine its ability to scavenge ·O₂.

Hydrogen peroxide was produced in vitro by the action of glucose oxidase (0.15 U/ml) on glucose (3.6 mM). The rate of H₂O₂ production in the reaction mixture was determined by monitoring the absorption at 505 nm during its oxidative coupling with 4-aminopyridine and phenol to yield quinoneimine dye. The difference in the change of absorbance during the production of quinoneimine dye in the presence and absence of catalase (100 mg/ml) was considered as a conservative estimate of the rate of H₂O₂ production. DMTU (1 to 10 mM) was included in the reaction mixture to determine its ability to scavenge H₂O₂.

Determination of DMTU kinetics. To determine the pharmacokinetics of DMTU, two conscious dogs were given DMTU (500 mg/kg) as an intravenous bolus, and plasma samples were obtained 30 min and 1, 2, 3, 4, 6, 10, 12, 24, 30, 51, 72, and 98 hr thereafter. Pharmacokinetic data were analyzed by iterative nonweighted linear least-squares regression analysis.

Experimental preparation. Mongrel dogs of both sexes weighing 17 to 31 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg), intubated, and ventilated with room air by a Harvard ventilator. Ventilatory variables were adjusted on the basis of arterial blood gas determinations to maintain normal pH and adequate oxygenation. The chest was opened through the left fifth intercostal space and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated and encircled with a snares at a site distal to the first major diagonal branch. A Doppler flow probe was placed around the vessel distal to the snares. Polyethylene catheters were inserted through the left carotid artery into the aorta and through the left atrial appendage into the left atrium and were connected to Statham P23Db pressure transducers. A No. 8F Millar pressure transducer was introduced into the left ventricular cavity via an apical stab wound. The first derivative of left ventricular pressure (dP/dt) was obtained by electronic differentiation. A Doppler ultrasonic wall thickening probe was positioned over the portion of the left ventricle to be rendered ischemic, and a second probe was placed on the posterior left ventricular wall to serve as a control. The Doppler probe consists of a 4 mm ultrasonic crystal bonded to a 1.5 cm-diameter fabric disk impregnated with silicone rubber (Silastic). The disk was sutured to the epicardium with 6-0 prolene stitches penetrating 0.5 to 1.0 mm into the myocardium, thus producing minimal trauma. Aortic pressure, left ventricular pressure and dP/dt, left atrial pressure, LAD blood flow velocity, myocardial thickening, and the electrocardiogram were recorded on an eight-channel, direct-writing oscillograph (Gould Brush system 200).

Experimental protocol. After baseline recordings, dogs received either DMTU (Aldrich Chemical Co.), 500 mg/kg in 40 ml of normal saline intravenously over 45 min starting 30 min before coronary artery occlusion, or an equal volume of vehicle (normal saline). This dose of DMTU was selected because it is similar to the dosages previously shown to afford protection in other models of free radical–mediated damage and because it resulted in DMTU plasma levels comparable to those shown to scavenge ·OH in vitro. Moreover, in pilot studies, higher doses were found to cause a decline in arterial pressure. The LAD was occluded for 15 min, followed by reperfusion. Complete restoration of flow was confirmed by the Doppler flow probe measurements. No attempt was made to resuscitate animals that developed ventricular fibrillation during ischemia or reperfusion. Hemodynamic and wall thickening data were obtained 35 min before coronary occlusion (before treatment), 5 min before coronary occlusion (25 min after start of treatment), 5 min after coronary occlusion, and 30 min and 1, 2, 3, and 4 hr after reperfusion. At the end of the study, the dogs were given heparin (6000 U) followed by a lethal dose of potassium chloride, and the hearts were excised.

Determination of occluded bed size. The size of the occluded coronary vascular bed was determined by a previously described postmortem perfusion technique. Briefly, two canulas were inserted into the LAD, one immediately proximal and the other immediately distal to the site of the previous occlusion. The distal LAD was perfused with normal saline. The proximal LAD and circumflex arteries were perfused with a 0.5% solution of monastral blue dye in saline while the left coronary ostium was occluded with the investigator’s finger. The two vascular beds were perfused simultaneously for 2 min.
at equal physiologic pressure (100 mm Hg) to prevent flow across collateral channels. The heart was then cut into 1 cm thick slices in a plane parallel to the atrioventricular groove, and all atrial, valvular, and right ventricular tissue was excised. The slices were incubated in a 1% solution of triphenyltetrazolium chloride (TTC) for 20 min at 37°C to verify the absence of infarction. In viable myocardium, TTC is converted by dehydrogenases to a red formazan pigment that stains tissue dark red; in necrotic myocardium, however, such staining does not occur because of the loss of dehydrogenases. The ability of this technique to identify irreversibly injured myocardium within hours from the onset of ischemia, particularly when followed by reperfusion, has been previously demonstrated. The portion of the left ventricle supplied by the previously occluded coronary artery (occluded bed) was identified by the absence of blue dye and separated from the rest of the left ventricle. Both components were weighed to determine occluded bed size as a percentage of left ventricular weight.

Regional myocardial blood flow. Quantification of collateral flow during coronary occlusion is important because the severity of contractile dysfunction after reperfusion is closely related to this variable. Accordingly, random differences in collateral flow may result in substantial differences in recovery of function between control and treated groups, which could be ascribed erroneously to a drug effect. We therefore determined regional myocardial blood flow to the ischemic zone by the radioactive microsphere technique 10 min after coronary occlusion. Microspheres (15 ± 3 μm [mean ± SD] in diameter; Dupont Co.) were obtained as 1 mCi of nuclide suspended in 10 ml of 10% dextran with 0.01% Tween 80 to minimize clumping. Microspheres were labeled with 99mTc or 141Ce. Before injection, the vial containing microspheres was vigorously agitated on a mechanical mixer for 2 min. Approximately 1.5 million spheres were then injected into the left atrium in 3 ml of normal saline over 10 to 15 sec; the catheter was flushed with an additional 10 ml of saline. Beginning 15 sec before and continuing for 2 min after the end of the injection, blood was withdrawn from the aorta with a Harvard pump at a constant rate of 4.05 ml/min. After postmortem perfusion and TTC staining, four transmural samples (1.0 to 1.5 g) were obtained from both the occluded and nonoccluded beds. Each specimen was divided into epicardial and endocardial halves and weighed. To avoid admixture of ischemic and nonischemic tissue, ischemic samples were obtained by cutting the margins of the unstained region. The radioactivity of the tissue and reference blood samples was determined with a sodium iodide crystal well counter. Regional myocardial blood flow was calculated by standard methods as previously described.

Regional myocardial function. Regional myocardial function was assessed with a pulsed Doppler epicardial wall thickening probe, as previously described. In brief, the pulsed Doppler technique uses a single epicardial transducer to determine systolic myocardial wall displacement by digitally integrating the velocity of myocardial layers passing through the range-gated sample volume. Representative examples of the tracings obtained with this probe are shown in figure 1. The beginning and end of systole were determined from the onset of the rapid upstroke of the left ventricular pressure tracing and the peak negative dP/dt, respectively. Systolic thickening fraction was calculated by dividing net systolic thickening by end-diastolic wall thickness as determined by the range gate depth. Net systolic thickening was defined as the maximal systolic increase in wall thickness from the end-diastolic value. When paradoxical wall thinning persisted for 50% or more of systole, the maximal extent of wall thinning was subtracted from wall thickening to give net systolic thickening. Because wall thickening can vary markedly with the respiratory cycle, all measurements were taken at end-expiration. At least 6 beats were averaged at each time point. Thickening fraction determined by the Doppler method correlates closely with thickening fraction measured with two ultrasonic transit-time crystals.

The use of the Doppler probe is advantageous, however, in that the single epicardial crystal eliminates the trauma of intramyocardial crystal insertion and the potential artifacts resulting from misalignment of two sonomicrometers.

Determination of DMTU plasma levels. To compare the DMTU plasma levels attained in vivo with the concentrations of this compound that scavenge ·OH in vitro, two additional open-chest dogs were given DMTU in the same dosage administered to the experimental group (500 mg/kg iv over 45 min). Plasma samples for DMTU determination were obtained 30, 45, 60, 105, 165, and 285 min after the start of the infusion.

Measurement of DMTU concentration. DMTU concentration was measured by gas chromatography at the University of Colorado Health Sciences Center. An internal standard was made by dissolving diethyl sulfone (3.5 g) (ICN Pharmaceutical, Inc., Plainville, NY) in deionized water (100 ml) in a volumetric flask. The solution was then filtered through a Whatman No. 2 filter and stored at room temperature. An aliquot of this solution (1%) was added to a 1 ml test sample. The test sample was then treated with perchloric acid (1.8M) to precipitate the protein (200 μl perchloric acid/ml sample). Each sample was vortexed and placed on ice for 10 min, then centrifuged at 500 g for 5 min. To the supernatant was added 32M K2HPO4 (200 μl). The sample was vortexed, placed on ice for 5 min, and centrifuged at 500 g for 5 min. One microliter of the supernatant was injected into the gas chromatograph. DMTU concentration was assessed with a Varian gas chromatograph (Model 3700) equipped with an FID detector and a Varian 4270 integrator. The 2 ft long, 1/4 inch od × 2 mm id glass column was packed with 20% Carbowax 20M on Supelcoport, 80/100 mesh (Supelco, Bellfonte, VA). Operating conditions were: injector temperature 260°C; oven temperature initially 120°C for 2 min, temperature program 30°C/C/min to 155°C for 4 min. Helium, hydrogen, and air flows were set at 30, 30, and 300 ml/min, respectively. The integrator was programmed to integrate and print out peak area and retention time. The peak area for DMTU was divided by the peak area for the internal standard to give a peak area ratio for each injection.

Statistical analysis. All values are reported as mean ± SEM. A repeated-measures analysis of variance was used to compare systemic hemodynamic variables and thickening fraction between control and treated dogs. The unpaired Student t test was used to compare values for arterial blood gases and hemoglobin, size of the occluded bed, and regional blood flow between control and treated dogs. A p value < .05 was considered statistically significant.

Results

Effect of DMTU on superoxide anion and hydrogen peroxide in vitro. The activity of xanthine oxidase (0.02 U/ml) on xanthine (53 μM) produced 4.20 nmol of superoxide dismutase–inhibitable ·O2− (in a total cuvette volume of 2.6 ml) as determined by cytochrome c reduction during the first 2 min after initiation of the reaction. DMTU (1 to 10 mM) did not alter the rate of ·O2− generation. Glucose oxidase (0.15 U/ml) acting on glucose (3.6 mM) produced 0.011 μmol H2O2/ml/min,
which was completely scavenged by 100 μg/ml catalase. Inclusion of DMTU (1 to 10 mM) had absolutely no effect on H₂O₂ production in this system. These results agree with those previously reported by others. On the other hand, it has been previously demonstrated that DMTU reduces ·OH concentration in vitro and that this effect is significant after addition of 5 mM DMTU.

Plasma levels of DMTU. Listed in table 1 are the plasma levels of DMTU in two open-chest dogs given 500
mg/kg over 45 min. Thirty minutes after initiation of the infusion (the time when the experimental group underwent coronary occlusion), DMTU plasma concentrations were 4.7 and 5.1 mM. DMTU levels continued to rise until the end of the 45 min infusion and peaked between 45 and 60 min after start of infusion (interval corresponding to the first few minutes of coronary reperfusion in the experimental group). DMTU plasma concentrations then declined slowly, and 4 hr after discontinuing the infusion (i.e., at the time when the dogs in the experimental group were killed) they still exceeded 4 mM. These data demonstrate that the dosage in this study resulted in relatively steady DMTU plasma levels that were equal to or greater than the concentrations that effectively scavenge ·OH in vitro (5 mM).

**Pharmacokinetics of DMTU.** The pharmacokinetic variables of DMTU in the dog are unknown. Because this information would be important in the design of future studies, additional experiments were conducted to determine the elimination half-life and volume of distribution of this agent. In the two conscious dogs in which DMTU plasma levels were measured for 96 hr after administration of a 500 mg/kg bolus, the elimination half-life of DMTU was found to be 28.7 and 33.7 hr, respectively, and the volume of distribution at steady state 1.09 and 1.53 liters/kg, respectively. The large volume of distribution provides further evidence for the ability of DMTU to penetrate the interstitial and intracellular compartments.

**Exclusions.** Of the 51 dogs initially anesthetized for the recovery of function protocol, four were not used because of hypoxemia (PaO₂ < 65 mm Hg) and two because of severe anemia (hemoglobin concentration < 7 g/dl). Four animals (two from each group) were excluded because of high collateral flow to the ischemic region (0.32 and 0.61 ml/min/g in the control and 0.33 and 0.57 ml/min/g in the treated dogs). Although these animals with mild ischemia exhibited dyskinesis 5 min after coronary occlusion, they showed little or no myocardial stunning; that is, contractile function returned to values similar to preocclusion levels within 1 hr of reperfusion (at 1 hr, thickening fraction, expressed as percent of preocclusion, was 80% and 93%, respectively, in the control dogs, and 80% and 85%, respectively, in the treated dogs). These data are consistent with our previous observations that collateral flow is a major determinant of the recovery of function after reperfusion, and emphasize the importance of measuring blood flow to the ischemic zone when investigating myocardial stunning. Two dogs (one in the control and one in the treated group) died of ventricular fibrillation during ischemia, and 10 died of ventricular fibrillation immediately after reperfusion (seven in the control and three in the treated group). Thus analysis of data was carried out in 15 control and 14 treated dogs.

**Arterial blood gases and hemoglobin.** Particular care was taken to ensure that acid-base balance and tissue oxygenation were adequate in every dog admitted to the study. As a result, arterial blood pH, Po₂, and hemoglobin concentrations were within normal limits in all control and treated animals. Thirty minutes before coronary artery occlusion, the following measurements were obtained in the control and treated groups, respectively: arterial pH, 7.42 ± 0.01 and 7.43 ± 0.01; arterial Po₂, 87 ± 4 and 87 ± 4 mm Hg; arterial hemoglobin concentration, 12.0 ± 0.4 and 12.9 ± 0.4 g/dl. Arterial blood gases were monitored during the rest of the experiment and were kept within normal limits (pH 7.36 to 7.44; Po₂ > 65 mm Hg) by adjusting ventilatory variables.

**Systemic hemodynamics.** In the treated group, measurements were obtained before and 25 min after start of DMTU infusion (i.e., 5 min before coronary occlusion [preocclusion values]). In the control group, preocclusion measurements were taken 5 min before coronary occlusion. Infusion of DMTU did not appreciably affect any of the measured hemodynamic variables (table 2). As shown in figure 2, there were no significant differences between control and treated groups with respect to preocclusion heart rate, mean arterial blood pressure, or peak positive left ventricular dP/dt. Preocclusion mean left atrial pressure was slightly lower in the treated group compared with the control group (4.7 ± 0.3 vs 6.6 ± 0.5 mm Hg; p < .05), but this difference became insignificant at subsequent time points. No significant hemodynamic difference developed between the two groups throughout the course of the study except for a modest decline in left atrial pressure at 4 hr of reperfusion in treated compared with control dogs (3.8 ± 0.1 vs 6.3 ± 1.0 mm Hg; p < .05) (figure 2).

### TABLE 1

<table>
<thead>
<tr>
<th>Time after start of infusion (500 mg/kg over 45 min)</th>
<th>Dog 1</th>
<th>Dog 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>4.7</td>
<td>5.1</td>
</tr>
<tr>
<td>45 min</td>
<td>7.8</td>
<td>7.1</td>
</tr>
<tr>
<td>60 min</td>
<td>9.0</td>
<td>6.8</td>
</tr>
<tr>
<td>105 min</td>
<td>6.4</td>
<td>5.3</td>
</tr>
<tr>
<td>165 min</td>
<td>5.6</td>
<td>4.9</td>
</tr>
<tr>
<td>285 min</td>
<td>5.6</td>
<td>4.5</td>
</tr>
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</table>
TABLE 2
Hemodynamic variables before and 25 min after start of DMTU infusion (mean \pm SEM)

<table>
<thead>
<tr>
<th></th>
<th>Before DMTU (preocclusion)</th>
<th>25 min after DMTU (preocclusion)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>115 \pm 5</td>
<td>109 \pm 5</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>143 \pm 5</td>
<td>147 \pm 4</td>
<td>NS</td>
</tr>
<tr>
<td>Mean left atrial pressure (mm Hg)</td>
<td>5.0 \pm 1.0</td>
<td>4.7 \pm 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic thickening fraction (%)</td>
<td>0.02</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>LAD territory</td>
<td>34 \pm 2</td>
<td>33 \pm 2</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic thickening fraction (%)</td>
<td>22 \pm 2</td>
<td>24 \pm 2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Occluded bed size and regional myocardial blood flow. The two groups were similar with respect to occluded bed size and coronary collateral flow (table 3). The occluded bed size was 28 \pm 1% of the left ventricle in the control group and 26 \pm 2% in the treated group. Transmural blood flow to the ischemic zone was 0.09 \pm 0.02 ml/min/g (range 0.02 to 0.27) in control dogs and 0.10 \pm 0.02 ml/min/g (range 0.04 to 0.23) in treated dogs. Transmural blood flow to the nonischemic zone was also similar (1.31 \pm 0.13 ml/min/g in control dogs and 1.14 \pm 0.07 ml/min/g in the dogs given DMTU). As a result, the ratio of transmural ischemic zone flow to transmural nonischemic zone flow (an index of the oxygen supply/demand ratio in the ischemic region) was indistinguishable in the two groups. Likewise, there were no discernible differences between control and treated animals with respect to endocardial or epicardial flow to either the ischemic or nonischemic regions.

Regional myocardial function. Systolic thickening fraction in the control (nonischemic) region tended to decrease somewhat in the course of the experiment but remained similar in the two groups throughout the study (table 4). Baseline systolic thickening fraction in the territory to be rendered ischemic averaged 29 \pm 2% in control and 33 \pm 2% in treated dogs (NS). The infusion of DMTU did not produce any appreciable change in thickening fraction (table 2). Figure 3 illustrates the results of serial measurements of systolic thickening expressed as a percent of preocclusion values. The two groups did not differ with respect to the extent of paradoxical systolic thinning during ischemia. Recovery of function after reperfusion, however, was consistently greater in treated animals throughout the 4 hr observation period. Analysis of variance showed a significant (p < .05) overall difference in systolic thickening fraction after reflow. Thickening fraction (expressed as percent of preocclusion values) was 9 \pm 14% in control vs 53 \pm 9% in treated dogs (p < .03) at 1 hr of reperfusion, 23 \pm 13% vs 55 \pm 9% (p < .05) at 2 hr, 28 \pm 14% vs 60 \pm 9% (p < .05) at 3 hr, and 36 \pm 13% vs 67 \pm 5% (p < .05) at 4 hr.
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TABLE 3
Occluded bed size and regional myocardial blood flow (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Occluded bed (g)</th>
<th>Ischemic zone flow (ml/min/g)</th>
<th>Nonischemic zone flow (ml/min/g)</th>
<th>IZF/NZF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Epidermal</td>
<td>Endocardial</td>
<td>Mean</td>
</tr>
<tr>
<td>Control (n = 15)</td>
<td>28 ± 1</td>
<td>0.15 ± 0.03</td>
<td>0.05 ± 0.01</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>DMTU (n = 14)</td>
<td>26 ± 2</td>
<td>0.12 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.10 ± 0.02</td>
</tr>
</tbody>
</table>

There were no significant differences between control and treated dogs.

*Ratio of transmural ischemic zone flow to simultaneous transmural nonischemic zone flow.

Representative examples of the time course of recovery of contractile function in the two groups are illustrated in figure 1.

**Histochemical analysis.** TTC staining confirmed the absence of infarction in all animals. This finding is consistent with previous studies showing that the myocardial injury associated with a 15 min coronary occlusion in the dog is completely reversible.

**Discussion**

This study demonstrates that DMTU produces a sustained improvement in recovery of myocardial function after regional reversible ischemia. After 1 hr of reperfusion, the previously ischemic myocardium had recovered approximately half of its baseline systolic function in dogs treated with DMTU but was still essentially akinetic in control animals. Over the ensuing 3 hr, the performance of the stunned myocardium in the treated group remained at a level approximately twice that of the control group. These results implicate ·OH (or a secondary radical derived from it) in the pathogenesis of regional postischemic dysfunction. Although other studies have suggested that ·OH may contribute to the dysfunction that follows prolonged global ischemia in the arrested heart perfused with artificial solutions, no previous investigation has suggested a role of the ·OH radical in the myocardial stunning observed in the working, blood-perfused heart after brief, reversible regional ischemia.

FIGURE 3. Systolic thickening fraction in the LAD territory 5 min after coronary occlusion (O) and at selected times after reperfusion in control (dashed line) and treated (continuous line) groups. Thickening fraction is expressed as percent of the values measured 5 min before coronary occlusion. Data are mean ± SEM. *p < .05 vs control.

TABLE 4
Systolic thickening fraction in the nonischemic zone (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Preocclusion*</th>
<th>Occlusion</th>
<th>30 min</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 15)</td>
<td>21 ± 2</td>
<td>24 ± 2</td>
<td>21 ± 2</td>
<td>20 ± 1</td>
<td>19 ± 2</td>
<td>19 ± 1</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>DMTU (n = 14)</td>
<td>24 ± 2</td>
<td>29 ± 2</td>
<td>23 ± 2</td>
<td>22 ± 2</td>
<td>21 ± 2</td>
<td>22 ± 2</td>
<td>21 ± 2</td>
</tr>
</tbody>
</table>

*The preocclusion values reported for the treated group are the measurements taken 5 min before coronary occlusion, i.e., 25 min after start of DMTU infusion. These values were not different from those obtained under baseline conditions before treatment (table 1).
Mechanism of the beneficial effects of DMTU. A detailed analysis was carried out to exclude nonspecific effects unrelated to the ·OH-scavenging properties of DMTU. Several variables that might influence postischemic dysfunction were examined, but no differences were found between control and treated animals that could account for the observed enhancement in recovery of contractility. The two groups were quite similar with respect to arterial pH, P02, and hemoglobin concentration, occluded bed size, collateral flow, and systemic hemodynamics. Left atrial pressure was slightly lower in treated dogs at 4 hr of reperfusion, but this factor, if anything, would be expected to reduce wall thickening by reducing preload.

It is unlikely that the attenuation of stunning by DMTU was related to removal of ·O2 or H2O2 because in our experiments in vitro using the xanthine-xanthine oxidase reaction to generate ·O2 and the glucose oxidase reaction to generate H2O2, no scavenging action on either oxygen metabolite was observed even with concentrations of DMTU (10 mM) that exceeded those achieved in the plasma in our dogs (5 to 9 mM). This conclusion is further supported by previous studies in vitro using different assays,11 in which 10 mM DMTU failed to affect ·O2 production by neutrophils and reacted only very slowly with H2O2 (estimated rate constant = 0.1 M-1 sec-1). DMTU has also been reported to react with H2O2 in other systems,27 but because of the extremely low rate constant it is unlikely that removal of H2O2 is a major mechanism for the protective effects of this agent. It should also be pointed out that there are reasons to suspect that the damage caused by H2O2 is mediated primarily by its conversion to ·OH via the Fenton reaction.9 For example, it has been demonstrated that the ability of H2O2 to damage bacteria28, 29 and DNA30 is primarily dependent on its reduction to ·OH.

Present results and the mechanism of postischemic dysfunction. The cause for the prolonged contractile dysfunction observed after reversible ischemia remains uncertain. Proposed mechanisms include ATP depletion,2, 3 altered calcium homeostasis secondary to sarcoplasmic reticulum dysfunction,31 functional interruption of cardiac sympathetic nerves,32 delayed activation of the reperfused region,33 and heterogeneous impairment of myocardial perfusion.9 The role of these abnormalities in producing postischemic dysfunction remains to be elucidated.

We5, 7, 8 and others6, 8 have proposed that postischemic dysfunction after a transient coronary occlusion may be caused by the generation of cytotoxic oxygen-derived free radicals during ischemia and/or early reperfusion. This hypothesis is supported by the finding that myocardial stunning after a 15 min coronary occlusion is markedly attenuated by pretreatment with “anti-free radical” agents, such as superoxide dismutase and catalase,6-8 N-2-mercaptopropionylglycine,7 and allopurinol.8 The present data obtained with an unrelated scavenger provide further evidence to corroborate the hypothesis that toxic oxygen metabolites play a significant role in the genesis of myocardial stunning. In particular, this study suggests that postischemic dysfunction is mediated in part by the ·OH radical or one of its reactive products.

Potential sources of hydroxyl radicals in ischemic/reperfused myocardium. In vitro, the ·OH radical can be formed from ·O2 and H2O2 by the metal-catalyzed Haber-Weiss reaction, in which ·O2 acts to reduce a transition metal ion (such as Fe3++ or Cu2+), and H2O2 then interacts with the reduced metal ion in a Fenton reaction to form ·OH or a species with similar reactivity.10 In the absence of metal ions, the rate constant for the Haber-Weiss reaction is extremely low; however, Fe3++ or Cu2+ are thought to be available in vivo to catalyze this reaction.10 Although ·OH production in biological systems has not been demonstrated definitively, numerous studies provide indirect evidence for the participation of this radical in tissue injury.9, 10, 25, 26, 35-39

Normally, tissues are not exposed to appreciable concentrations of ·OH because the precursors of this radical are degraded by physiologic mechanisms (·O2 by superoxide dismutase and H2O2 by catalase and glutathione peroxidase). During myocardial ischemia and reperfusion, however, several processes could lead to accumulation of ·O2 and H2O2, which in turn could generate ·OH. Superoxide production may increase in the intracellular space as a result of arachidonic acid metabolism, autoxidation of various compounds, and impaired oxygen metabolism resulting from ischemia-induced mitochondrial damage and accumulation of reducing equivalents.40 In addition, the enzyme xanthine oxidase and activated neutrophils may generate significant quantities of oxygen metabolites in the endothelial cells and in the extracellular space, respectively.40-44

Previous studies of free radicals in myocardial ischemia. Mounting evidence implicates oxygen free radicals in myocardial injury associated with ischemia and reperfusion.9, 40, 41 Rao et al.45 observed increased production of free radicals and lipid peroxidation products early after coronary occlusion. Enzymatic free radical scavengers such as superoxide dismutase and catalase reduce the size of the infarction resulting from a tem-
porary coronary occlusion followed by reperfusion\textsuperscript{12, 46, 47} and improve recovery of myocardial function after prolonged hypothermic global ischemia\textsuperscript{48, 49} and ischemia in vitro.\textsuperscript{30} The xanthine oxidase inhibitor allopurinol also reduces infarct size.\textsuperscript{42, 43} Although in one report\textsuperscript{51} it was found to be ineffective. Moreover, the combination of superoxide dismutase and mannitol — an ·OH scavenger — enhances recovery of left ventricular function after hypothermic global ischemia.\textsuperscript{52}

Relatively few studies have specifically addressed the role of the ·OH radical. Shlafer et al.\textsuperscript{53} showed that the ·OH scavenger dimethyl sulfoxide preserves mitochondrial function after a 2 hr ischemic period in isolated hearts. Other studies have found that mannitol enhances recovery of global left ventricular function in isolated heart or cardiopulmonary bypass preparations of prolonged hypothermic cardioplegia\textsuperscript{24, 25} or normothermic ischemia\textsuperscript{26} and that these effects are independent of hyperosmolar actions.\textsuperscript{25, 26} Our data extend these observations to the dysfunction that occurs after brief regional ischemia in the intact animal and thus provide evidence that the ·OH radical may play a significant role in the setting of transient coronary occlusion.

This concept is compatible with a number of recent studies in vitro. Using isolated hearts subjected to hypoxia, Myers et al.\textsuperscript{53} observed that creatine kinase release upon reoxygenation was reduced by deferoxamine, which suggests that hypoxia-induced myocardial damage is caused in part by ·OH. Blaustein et al.\textsuperscript{54} have shown that the contraction of isolated rat papillary muscles is depressed by addition of purine and xanthine oxidase and that this effect is prevented by catalase but not by superoxide dismutase, suggesting that the observed impairment of mechanical function is mediated by H$_2$O$_2$ and/or ·OH. Recently, Jackson et al.\textsuperscript{55} have shown that oxy-radicals generated by electrolysis depress the mechanical function of isolated rabbit hearts; this effect was markedly enhanced by iron, whereas the ·OH scavenger dimethyl sulfoxide exerted protective actions.

DMTU. We elected to use DMTU for several reasons. First, as demonstrated by our present results and by previous studies,\textsuperscript{11, 12} this agent is an effective scavenger of ·OH but not of O$_2$ and H$_2$O$_2$, at least at the plasma concentrations attained in this experiment. Consequently, the effects of DMTU should reflect the cytotoxic action of ·OH rather than that of O$_2$ or H$_2$O$_2$. Second, this compound is highly permeable\textsuperscript{11} and therefore may scavenge oxygen metabolites both intracellularly and extracellularly. Third, DMTU is considerably more effective than other ·OH scavengers such as mannitol, ethanol, and dimethyl sulfoxide.\textsuperscript{11, 56} For example, dimethyl sulfoxide, which is several times more reactive than either mannitol or ethanol,\textsuperscript{56} must be used in concentrations of 280 mM to provide the same degree of protection to bacteria against ·OH as is afforded by DMTU in concentrations of 5 mM.\textsuperscript{28} A concentration of DMTU of 10 mM inhibits ·OH-mediated methane production from dimethyl sulfoxide by 85%, whereas the same concentration of mannitol exerts no inhibition.\textsuperscript{11} Fourth, DMTU does not interfere with leukocyte functions other than ·OH generation.\textsuperscript{11} Finally, our pharmacokinetic data demonstrate that DMTU has a relatively long plasma half-life. This property is rather unique among free radical scavengers and would be useful in studies requiring prolonged protection against free radical–mediated damage.

DMTU has been shown to protect against a number of pathologic processes mediated by reactive oxygen metabolites, including pulmonary edema induced by activated neutrophils\textsuperscript{11, 27} or by an O$_2^*$-generating system (purine-xanthine oxidase),\textsuperscript{57} pulmonary oxygen toxicity,\textsuperscript{11} and radiation mortality.\textsuperscript{12} Since ·OH is the principal oxidizing radical generated by ionizing radiation,\textsuperscript{49} protection against radiation provides strong evidence for an ·OH-scavenging action of DMTU in vivo. The present study is the first to use DMTU in the setting of myocardial ischemia. Because of the beneficial effect on postischemic dysfunction, the favorable pharmacokinetic properties, the absence of appreciable hemodynamic actions at this dosage, and the other properties described above, DMTU appears to be a useful probe for assessing the role of ·OH in experimental cardiovascular studies.

**Clinical implications.** The results of this study provide a basis for developing therapeutic strategies aimed at preventing postischemic dysfunction. There are numerous clinical settings in which the myocardium is exposed to transient ischemia, such as unstable or variant angina, acute myocardial infarction with early reperfusion (spontaneous or induced by thrombolytic therapy), open heart surgery, and cardiac transplantation. The presence of myocardial stunning in these situations is suggested by recent observations\textsuperscript{3} and may be an important factor precipitating cardiac failure.\textsuperscript{58} Our results imply that effective reduction of postischemic myocardial dysfunction may be obtained with pharmacologic manipulations that either scavenge ·OH or prevent its generation.

The technical assistance of Jennifer Pocius, Terrence Edwards, Curtis Mills, Betty Washington, and Elaine M. Berger...
and the expert secretarial assistance of Megan White and Kathy Marshall are gratefully acknowledged.

References


46. Jolly SR, Kane WJ, Bailie MB, Abrams GD, Lucchesi BR: Canine
myocardial reperfusion injury: its reduction by the combined administration of superoxide dismutase and catalase. Circ Res 54: 277, 1984


51. Reimer KA, Jennings RB: Failure of the xanthine oxidase inhibitor allopurinol to limit infarct size after ischemia and reperfusion in dogs. Circulation 71: 1069, 1985


Attenuation of dysfunction in the postischemic 'stunned' myocardium by dimethylthiourea.
R Bolli, W X Zhu, C J Hartley, L H Michael, J E Repine, M L Hess, R C Kukreja and R Roberts

*Circulation*. 1987;76:458-468
doi: 10.1161/01.CIR.76.2.458

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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