Reduction of Canine Myocardial Infarct Size by a Diffusible Reactive Oxygen Metabolite Scavenger

Efficacy of Dimethylthiourea Given at the Onset of Reperfusion

Frank P. Carrea, Edward J. Lesnefsky, John E. Repine, Robert H. Shikes, and Lawrence D. Horwitz

A number of scavengers of reactive oxygen metabolites reduce myocardial injury when given before ischemia and reperfusion, but few, if any, have proven to be effective when given near the onset of reperfusion. This is particularly true when infarct size is measured after at least 48 hours of reperfusion, when the full extent of myocardial damage has become apparent. Dimethylthiourea (DMTU) is an extremely diffusible, potent scavenger of hydroxyl radical, hydrogen peroxide, and hypochlorous acid, with a long half-life of 43 hours. Sixteen chloralose-anesthetized dogs underwent 90 minutes of left anterior descending coronary artery (LAD) occlusion followed by 48 hours of reperfusion. Collateral flow was measured by radioactive microspheres. Infarct size and risk area were measured by a postmortem dual-perfusion technique using triphenyl tetrazolium chloride and Evan’s blue dye. In eight dogs, therapy with DMTU (500 mg/kg i.v.) was given during the last 15 minutes of ischemia and the first 15 minutes of reperfusion. In eight control dogs, the same volume of 0.9% saline was given during the last 15 minutes of ischemia through the first 15 minutes of reperfusion. Infarct size as a percent of risk area was reduced in the DMTU-treated group compared with the saline-treated controls (DMTU=42±4% versus saline=59±4%, p<0.01). There were no differences between the groups in risk area as a percent of the left ventricle (DMTU=25±2% versus saline=21±2%, p=NS), in LAD endocardial flow during ischemia (DMTU=3.5±0.4 versus saline=4.0±0.5 ml/100 g/min, p=NS), in LAD transmural flow during ischemia (DMTU=13.3±1.3 versus saline=12.0±1.5 ml/100 g/min, p=NS), or in the heart rate-blood pressure product. Histological and in vitro biochemical analyses confirmed that the tetrazolium method accurately delineated infarct size in saline- and DMTU-treated dogs. Thus, DMTU given near the onset of reperfusion dramatically reduced infarct size measured after an interval sufficient for irreversibly damaged cells to become necrotic. The efficacy of DMTU in reducing infarct size may reflect its rapid entry into myocardial cells as well as continued protection for a prolonged period during reperfusion. (Circulation Research 1991;68:1652-1659)

There has been considerable concern that early reperfusion of ischemic myocardium, which terminates damage due to ischemia, may cause further damage through a different mechanism, resulting in suboptimal myocardial salvage.1,2 This "reperfusion injury" appears to be mediated by the formation of reactive oxygen metabolites (including H$_2$O$_2$, -O$_2^-$, OH$, and others) that are toxic to myocardial cells.6-8 Analysis by spin-trap techniques has generally concluded that a large burst of reactive oxygen metabolites is produced immediately after hypoxic or ischemic myocardium is reoxygenated.5,9 However, another study10 has observed production of reactive oxygen metabolites for several hours after the onset of reperfusion.

Therapeutic strategies have focused on treatment with enzymatic scavengers of reactive oxygen metabolites or with iron chelators in animal models of ischemia and reperfusion.11-15 Most of these agents have serum half-lives on the order of minutes16 and do not readily cross cell membranes. Administration...
of a superoxide anion scavenger, superoxide dismutase, has generally resulted in short-term myocardial salvage, but when reperfusion is carried out for longer periods (24 hours to 7 days), myocardial salvage has not been consistently observed. 

This discrepancy between short- and long-term results may be due to at least two factors. Continued production of reactive oxygen metabolites during the first 24 hours of reperfusion could cause continuing damage during this period. This ongoing production probably extends well beyond the time in which many agents demonstrate active scavenging activity. Alternatively, the methodology used to determine myocardial infarct size (tetrazolium staining) may fail to identify all irreversibly injured tissue early in reperfusion, leading to an underestimation of the amount of necrosis that will eventually occur.

Another disappointing aspect of efforts to reduce reperfusion injury has been that agents that appear to reduce myocardial injury if given before ischemia have generally failed to do so if they are given near the onset of reperfusion. This is troubling, since clinical application of an intervention to prevent reperfusion injury after acute myocardial infarction would involve administration after ischemia is well established. The poor results demonstrated by most agents with administration after ischemia has begun may hinge on their ability to enter cells. Antioxidant scavenger enzymes, such as catalase and superoxide dismutase, or the iron chelator, deferoxamine, do not readily enter myocardial cells, particularly when regional myocardial perfusion is diminished.

A pharmacological intervention that is useful in preventing reperfusion injury by reactive oxygen metabolites should ideally be capable of entering myocardial cells rapidly to counter the burst of reactive oxygen metabolites generated early in reperfusion. It should also maintain sufficient myocardial levels to afford protection against low levels of oxidant production during the subsequent hours. Dimethylthiourea (DMTU) is an agent that is highly diffusible, has a long half-life, and is an effective scavenger of hydrogen peroxide, hydroxyl radical, and hypochlorous acid. In this study, we administered DMTU after regional myocardial ischemia was well established and were able to demonstrate a 30% reduction in infarct size at 48 hours of reperfusion.

**Materials and Methods**

**Instrumentation**

Nineteen male mongrel dogs (25.8±0.9 kg) were anesthetized with thiamyl sodium (20 mg/kg i.v.) followed by α-chloralose (100 mg/kg i.v. supplemented as needed). The dogs were intubated and ventilated with a respirator (Harvard Apparatus, South Natick, Mass.) using a mixture of 35% oxygen and 65% nitrogen, which maintains arterial blood gases in the usual physiological range at Denver's altitude.

By use of a sterile technique, a left thoracotomy was performed. The heart was suspended in a pericardial cradle. Catheters were inserted into the proximal descending aorta and left atrium. A snare occluder was placed loosely around the left anterior descending coronary artery (LAD) distal to its first diagonal branch (2–3 cm from the LAD origin).

**Experimental Protocol**

During a 15-minute postinstrumentation equilibration period, an injection of 0.2 ml Tween 80 in 2 ml of 0.9% saline was performed to test for adverse hemodynamic effects. Before LAD occlusion, 1–2 million 15-μm-diameter radiolabeled microspheres were injected into the left atrium for a baseline regional myocardial blood flow measurement. Subsequently, a 30-second test occlusion was performed. Epicardial cyanosis was apparent in all dogs during the test occlusion.

Aortic and left atrial pressures were recorded using Statham P23Db transducers (Gould, Cleveland, Ohio). The lead II electrocardiogram was recorded with subcutaneous needle electrodes. Blood pressure, left atrial pressure, and a lead II electrocardiogram were continuously monitored on an oscillograph (model R 612, Beckman Instruments, Inc., Fullerton, Calif.). The heart rate–blood pressure product was calculated by multiplying heart rate times mean aortic pressure.

Complete blood counts were obtained from arterial samples before occlusion, at the end of ischemia, at 2 hours of reperfusion, and at 48 hours of reperfusion. Tidal volume, respiratory rate, and end-expiratory pressure were adjusted to maintain a physiological pH and PO₂.

LAD ligation was performed by tightening the snare for 90 minutes. Epicardial cyanosis was apparent in all dogs. At 60 minutes of occlusion, a second set of microspheres was injected into the left atrium. The treatment phase of the protocol was begun at 75 minutes of occlusion. Dogs were randomized to receive either DMTU (500 mg/kg i.v. in 100 ml of 0.9% NaCl) or saline (100 ml of 0.9% NaCl) during the last 15 minutes of ischemia and the first 15 minutes of reperfusion. After 90 minutes of ischemia, the LAD occluder was released. A third set of microspheres was injected into the left atrium at 2 hours of reperfusion. The left atrial and aortic lines were externalized using subcutaneous tunnels, and the chest was closed using sterile technique. Ampicillin (500 mg i.v.) was administered as a prophylactic antibiotic to all dogs. Assisted ventilation was continued until adequate spontaneous respirations returned.

The dogs were returned to the kennel, where they were fed standard chow for 48 hours. Morphine sulfate was administered intramuscularly as deemed appropriate for pain control. On day 2, the dogs were again anesthetized with thiamyl sodium (20 mg/kg i.v.) followed by α-chloralose (100 mg/kg i.v.). Hemodynamics were recorded, and blood samples were obtained for complete blood counts and arterial blood...
gases. A fourth injection of microspheres was then performed. Subsequently, the thoracotomy incision was reopened. Heparin (5,000 IU i.v.) was then administered, and the heart and proximal aorta were removed.

Measurement of Infarct Size

The heart and aorta were connected to a dual-perfusion apparatus. The aortic root and LAD immediately distal to the site of prior occlusion were cannulated. The proximal LAD was ligated at the site of prior occlusion. This allowed perfusion of the circumflex territory with Evan’s blue dye through the aortic root while the LAD territory was perfused through the LAD catheter with a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) at 37°C. Perfusion pressure was equal in both canuluses and was maintained at 100 mm Hg.23-25

After 5 minutes, the heart was removed from the perfusion apparatus. The left ventricle was isolated and weighed. Five to seven transverse sections of ~1-cm thickness were then obtained by sectioning parallel to the atroventricular groove. The slices were weighed and traced onto clear acetate sheets to demarcate 1) remote normal (blue stain), 2) ischemic, noninfarct (TTC-positive, red stain), or 3) ischemic, infarct (TTC-negative, unstained).

Regional Myocardial Blood Flow

Regional myocardial blood flow was assessed before occlusion, at 75 minutes of occlusion, at 60 minutes of reperfusion, and at 48 hours of reperfusion using 15-μm-diameter microspheres (Dupont, Wilmington, Del.) containing either 57Co, 40Sc, 85Sr, or 113Sn.15 One to 2 million microspheres in a 0.01% Tween 80 suspension (in 10% dextran) were diluted to a final volume of 2 ml with 0.9% NaCl. This suspension was agitated vigorously, injected through the left atrial catheter, and flushed in with 6 ml of 0.9% NaCl. Starting at 10 seconds before injection of the microspheres and continuing until 3 minutes after the injection, a reference sample was withdrawn from the aortic catheter at a rate of 2 ml/min.15 This procedure was repeated at each time point using a different isotope.

After the hearts were perfused with dye and sliced, each slice was divided into regions of nonischemic (blue) and ischemic (red and white) flow, based on staining characteristics. These were then sectioned into endocardial, midmyocardial, and epicardial zones. At least two samples (0.5–1.5 g) from each zone were then separated and weighed for radionuclide counting in a three-channel gamma spectrophotometer (model 5000, Packard Instrument Co., Inc., Meriden, Conn.) using appropriate energy windows.15 Background and crossover counts from other isotopes were accounted for, and blood flow measurements (ml/100 g/min) were obtained. To avoid any error from apparent microsphere leakage or tissue edema, flows to the ischemic zone segments during ischemia and day 1 reperfusion were multiplied by a correction factor. This factor was calculated as the ratio of preocclusion blood flow in the nonischemic myocardial segment to the preocclusion blood flow in a segment subsequently made ischemic.26

Serum DMTU Analysis

Plasma samples were obtained before and at the end of the 30-minute DMTU infusion and at 2 hours of reperfusion, 24 hours of reperfusion, and 48 hours of reperfusion for subsequent analysis of DMTU content by high-performance liquid chromatography. The plasma samples were centrifuged in a microfuge (Amicon, Beverly, Mass.) with 500–molecular weight filters at 8,500 rpm (6,600g) for 90 minutes. This filtrate contains most of the plasma liquid plus all free components with <500 molecular weight. Aliquots of an internal standard, 0.1% dimethyl sulfoxide, were added to the plasma samples.

Ten-microliter samples were then injected in a mobile phase of deionized water by an autosampler (model 9095, Varian Associates, Inc., Palo Alto, Calif.) using a pump (model 510, Waters Instruments, Inc., Rochester, Minn.). Each sample was passed through a Hypersil ODS, 5-μm, 15-cm column (Jones Chromatography USA, Inc., Littleton, Colo.) to a variable wavelength spectrophotometer (model 481, Waters Instruments) set at 230 nm. The data were transferred to a data module (model 740, Waters Instruments). DMTU concentrations were determined using the DMTU/dimethyl sulfoxide ratio from a standard curve.27,28

Histopathology

The TTC stain is an established method of assessing viable versus infarcted tissue at times greater than 3–6 hours, although some antioxidant strategies are reported to alter the tissue-staining characteristics of TTC.20 Therefore, we tested the ability of TTC staining to distinguish viable, previously ischemic tissue from infarcted tissue in our preparation. Myocardium that remained after sections were removed for determination of myocardial blood flow was stored in 10% buffered formalin. Sections of myocardium from the center of TTC-positive (red), TTC-negative (unstained), and Evan’s blue–stained segments were randomly selected from three DMTU-treated dogs and three saline-treated dogs. These segments were labeled and reviewed in a blinded manner by a cardiac pathologist (R.H.S.). The segments were embedded in paraffin, and three serial sections were stained with hematoxylin and eosin, Masson’s trichrome, or phosphotungstic acid–hematoxylin. The sections were semiquantitatively assessed for coagulative necrosis, contraction-band necrosis, interstitial edema, hemorrhage, and interstitial neutrophil infiltration.

Presence of coagulative necrosis characterized by changes in staining qualities and loss of myocyte subcellular structural detail was scored as 0=none (normal), 1=mild, 2=moderate, and 3=severe. Presence of contraction band necrosis characterized by myocyte contraction bands was scored as 0=none (normal), 1=occasional, 2=moderate,
3=frequent. Interstitial edema was scored as 0=normal, 1=focal and mild loosening of interstitial connective tissue stroma, 2=diffuse and moderate loosening of interstitial connective tissue stroma, 3=diffuse and severe loosening of interstitial connective tissue stroma. Hemorrhage was scored as 0=absent, 1=focal and mild extravasation of red blood cells, 2=focal or diffuse but moderate extravasation of red blood cells, and 3=diffuse and severe extravasation of red blood cells. Neutrophil infiltration was scored as 0=absent, 1=present in a few high-power fields, 2=present in approximately half of high-power fields, and 3=present in most high-power fields.

In Vitro Assessment on the Lack of Effect of DMTU on TTC Staining

The TTC stain is a histochemical stain that requires dehydrogenase activity to reduce TTC to formazan. In addition to histological examination, we performed studies in vitro to exclude an effect of experimentally relevant concentrations of DMTU on TTC staining. The staining characteristics of TTC with and without addition of DMTU were assessed by modification of techniques reported by Singh et al.\textsuperscript{29} and by Green and Narahara.\textsuperscript{30} Approximately 500 mg of normal canine myocardium was homogenized (Polytron, Brinkmann Instruments, Inc., Westbury, N.Y.) in 5 vol of 10 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA. After centrifugation at 800g for 10 minutes at 4°C, the supernatant was used for enzymatic analysis. The basic assay materials were 0.05 ml of 20 mM TTC, 0.05 ml of 10 mM NaN\textsubscript{3}, and 0.2 ml tissue extract in 5-ml test tubes. Each assay condition required one of the following additions: phosphate buffer alone, 1 mM DMTU, 10 mM DMTU, 100 mM DMTU, 1 mg/ml NAD plus 100 mM DMTU, 0.1 ml sodium succinate, or 0.1 ml sodium succinate plus 100 mM DMTU. All test tubes were then brought to a final volume of 0.5 ml with 20 mM phosphate buffer (pH 7.5) and incubated at 37°C for 60 minutes. After incubation, 1.5 ml of 95% ethanol was added to each tube. After mixing thoroughly, they were centrifuged at 2,500g for 10 minutes at 4°C. The supernatants were then passed through a 0.22-μm filter (Millipore Corp., Bedford, Mass.). The absorbance of the filtrate at 458 nm, which represents formazan production, was recorded.

Statistical Analysis

Data reported are mean±SEM. All DMTU versus saline statistical comparisons at particular time points were performed using an unpaired t test. All within-group analyses over time were performed using a two-way analysis of variance. The Student-Newman-Keuls test was used for multiple comparisons.\textsuperscript{31} Collateral ischemic blood flow versus infarct size as a percent of risk area (MI/RISK) was assessed using an analysis of covariance (ANCOVA), with LAD endocardial (or transmural) blood flow during ischemia assigned as the independent variable and MI/RISK assigned as the dependent variable.\textsuperscript{25,32} A value of $p<0.05$ was considered significant.

Results

Study Group

Sixteen of the 19 dogs were included in the final analysis. Two dogs were excluded due to ischemic zone collateral flow $>30$ ml/100 g/min and failure to demonstrate an infarct by TTC (one DMTU- and one saline-treated dog). One dog (saline-treated) died between 8 and 16 hours after reperfusion and was excluded. Ischemic ST segment changes and reperfusion ventricular tachyarrhythmias occurred in all dogs included in the final analysis. During the early reperfusion period, ventricular fibrillation requiring defibrillation occurred in four DMTU-treated dogs and three saline-treated dogs.

Regional Myocardial Blood Flow

Ischemic zone endocardial blood flow during occlusion (DMTU=3.5±0.4 versus saline=4.0±0.5 ml/100 g/min, $p=NS$) and ischemic zone epicardial blood flow (DMTU=26.2±3.1 versus saline=23.7±2.2 ml/100 g/min, $p=NS$) were similar in both groups (Figure 1). Ischemic zone transmural blood flow during ischemia was also similar (DMTU=13.3±1.3 versus saline=12.0±1.5 ml/100 g/min, $p=NS$). Blood flows were not significantly different between the DMTU and saline groups at any time. Substantial and similar ischemia was produced by LAD ligation in both groups.
Myocardial Infarct Size

Infarct size (MI/RISK) and risk area (RISK/LV) are shown in Figure 2. MI/RISK was reduced by DMTU therapy compared with saline (DMTU=42±4% versus saline=59±4%, \( p<0.01 \)). MI/RISK versus transmural ischemic zone flow is shown in Figure 3. DMTU-treated dogs had decreased MI/RISK as a function of LAD endocardial or transmural ischemic flow by ANCOVA (both \( p<0.05 \)). Risk area as a percentage of the total left ventricle was similar in both groups (DMTU=25±2% versus saline=21±2%, \( p=\text{NS} \)). Total left ventricular mass was similar in both groups (DMTU=136.1±7.7 g versus saline=135.7±6.7, \( p=\text{NS} \)). Thus, there was a significant reduction in infarct size after DMTU treatment that did not appear to be influenced by differences in left ventricular mass, risk area, or ischemic coronary collateral blood flow.

Hemodynamics

There were no significant differences between the DMTU and saline groups at any time during the study in rate–pressure product, heart rate, mean arterial pressure, or left atrial pressure. The rate–pressure product at 48 hours was lower in each group than during the measurements obtained on the initial day of the study. The mean values for the rate–pressure product are shown in Figure 4. We conclude that DMTU caused no apparent hemodynamic effect that could explain its effect on myocardial infarct size.

Hematologic Parameters

The hematologic parameters are summarized in Table 1. Hemoglobin levels did not differ between the two groups and did not change during the study. White blood cell counts were similar in both groups at all times but did show a trend toward increased values after 48 hours of reperfusion.

DMTU Levels

DMTU levels were 6.2±0.1 mM at the end of the infusion, 4.8±0.2 mM at 2 hours of reperfusion, 3.0±0.6 mM at 24 hours of reperfusion, and 2.2±0.7 mM at 48 hours of reperfusion (Figure 5). Assuming first-order kinetics, the serum half-life is 43.7 hours.

Lack of Effect of DMTU on TTC Staining

The reduction of TTC to formazan as quantified by absorbance at 458 nM is shown in Table 2. There was neither inhibition nor accentuation of absorbance at 458 nM by experimentally relevant levels of DMTU. This suggests that DMTU does not artifactualy influence the conversion of TTC to formazan in vitro. Thus, the observed differences in infarct size are unlikely to be due to an effect of DMTU on the TTC staining characteristics of canine myocardium.
**Histopathology**

The results of the histopathologic scoring are shown in Table 3. In both groups, there were no abnormal histological findings in the ischemic–viable (TTC-positive [red]) or normal remote zones (Evan’s blue). Tissue that was identified as infarcted by lack of TTC staining had coagulation necrosis, contraction-band necrosis, interstitial edema, hemorrhage, and neutrophil infiltration. There were no significant differences within infarcted tissue when the DMTU- and saline-treated groups were compared. Thus, TTC staining accurately delineated infarcted from viable tissue at 48 hours according to our histological analysis.

**Discussion**

DMTU is a derivative of thiourea but does not precipitate the pulmonary edema associated with thiourea and has no cardiac toxicity in intact animals.\(^{22}\) It is a highly diffusible, low molecular weight compound that readily enters myocardium and other tissues in vivo.\(^{22,23}\) We found that DMTU has a serum half-life of 43 hours in our canine model. In previous reports, DMTU reduced reactive oxygen metabolite–mediated injury in lung,\(^ {22,34}\) kidney,\(^ {35}\) brain,\(^ {36}\) and skeletal muscle.\(^ {37}\) Other investigators have observed diminished canine myocardial stunning with DMTU.\(^ {38-40}\)

We previously reported that DMTU administration at the end of a 90-minute ischemic period reduced infarct size measured at 3 hours of reperfusion by 40%.\(^ {34}\) However, recent work has cast doubt on the accuracy and meaning of TTC delineation of infarct size with such a short reperfusion period. There is reason to believe that some tissue that appears to be viable by TTC staining very early in reperfusion may be irreversibly injured and will undergo necrosis by 24 hours of reperfusion.\(^ {15,17-19}\) It is also possible that some tissue that is viable at 3–6 hours of reperfusion may be subsequently injured over 24–48 hours of reperfusion by further generation of reactive oxygen metabolites.\(^ {10}\) Therefore, we decided to reevaluate the efficacy of DMTU in preventing injury during ischemia and reperfusion by studying myocardial infarction size after 48 hours of reperfusion. We chose this time point to avoid doubts regarding the reliability of TTC staining and because it permitted detection of late injury due to ongoing generation of reactive oxygen metabolites.

We found that peripheral intravenous infusion of DMTU near the onset of reperfusion produced substantial improvement in myocardial infarct size measured at 48 hours. This protection was apparent at all levels of collateral blood flow and was not due to changes in hemodynamic parameters during ischemia, white blood cell counts, or tissue neutrophil infiltration. There were no alterations in regional myocardial blood flow. DMTU did not cause artifactual changes in TTC staining characteristics. The histopathologic examination confirmed that TTC staining accurately distinguished necrotic from viable tissue at 48 hours.

**TABLE 1. Hematologic Parameters in Ischemic/Reperfused Dogs**

<table>
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<th>Absorbance at 458 nM</th>
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<tbody>
<tr>
<td>WBC ((×10^9/mm^3))</td>
<td>62.5±1</td>
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<tr>
<td>Baseline</td>
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<tr>
<td>Occlusion ((90 \text{ min}))</td>
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<td>Reperfusion ((120 \text{ min} \text{ and } 48 \text{ hr}))</td>
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<td>DMTU</td>
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<td>10.1±1.1</td>
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<td>17.1±1.6</td>
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<td>Saline</td>
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<td>17.6±4.1</td>
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<td>18.1±2.6</td>
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Hgb \((g/dl)\)

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<td>DMTU</td>
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<td>Saline</td>
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<td>12.4±1.0</td>
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Values are mean±SEM. WBC, white blood cell count; DMTU, dimethylthiourea-treated dogs; Saline, saline-treated (control) dogs; Hgb, hemoglobin. All \(p=\text{NS}\) DMTU vs. saline.

**TABLE 2. Reduction of 2,3,5-Triphenyltetrazolium Chloride to Formazan Quantified by Absorbance at 458 nM**

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<tr>
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<th>Absorbance at 458 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue extract alone</td>
<td>62.5±1</td>
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<tr>
<td>Tissue extract</td>
<td></td>
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<tr>
<td>Plus 1 mM DMTU</td>
<td>62.0±0.8*</td>
</tr>
<tr>
<td>Plus 10 mM DMTU</td>
<td>65.0±3.0*</td>
</tr>
<tr>
<td>Plus 100 mM DMTU</td>
<td>61.0±0.8*</td>
</tr>
<tr>
<td>Plus 1 mg/ml NAD and 100 mM DMTU</td>
<td>64.5±1.0*</td>
</tr>
<tr>
<td>Plus succinate</td>
<td>150.0±1.5†</td>
</tr>
<tr>
<td>Plus 50 mM succinate and 100 mM DMTU</td>
<td>152±2.7‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM; \(n=4\). DMTU, dimethylthiourea.

\(^*p=\text{NS}\) and \(t p<0.05\) vs. tissue extract alone; \(\ddagger p=\text{NS}\) vs. tissue extract plus succinate.
Results with other antioxidant measures in preventing myocardial damage in similar models of ischemia and reperfusion have been controversial. Some recent studies17-19 with superoxide dismutase, a scavenger of superoxide anion, have failed to confirm the benefits reported in earlier work. Superoxide dismutase is poorly diffusible and may not enter cells readily.16,39 In addition, it has a very short half-life, limiting the duration of protection it affords. Bovine superoxide dismutase has not generally been efficacious in studies performed after 48 hours or more of reperfusion.18 Although human recombinant superoxide dismutase reduced TTC-measured infarct size in one study done at 48 hours of reperfusion,12 it has recently been reported that this preparation of the enzyme alters TTC staining characteristics, which could lead to inaccuracy in assessing damage with the dye.20 Superoxide dismutase conjugated with polyethylene glycol has a long half-life.16,25 Chi et al25 reported that polyethylene glycol–superoxide dismutase (1,000 units/kg) reduced myocardial infarct size in a canine model of 6 hours of ischemia followed by 24 hours of reperfusion but that regular superoxide dismutase was ineffective. Tamura et al41 using the same dose of polyethylene glycol–superoxide dismutase in a canine model of 90 minutes of ischemia, demonstrated protection at 6 hours and 96 hours of reperfusion. However, Tanaka et al42 reported that an unusually high dose of polyethylene glycol–superoxide dismutase (10,000 units/kg) given together with catalase had no protective benefit in a canine model of 90 minutes of ischemia and 96 hours of reperfusion. It is possible that the higher dose may have been detrimental, since evidence has been published that manganese–superoxide dismutase may reduce damage in ischemic/reperfused rabbit hearts when given at low doses but exacerbate damage at higher doses.43

DMTU scavenges H$_2$O$_2$, -OH, and HOCl, but not -O$_2^-$ . It is possible that there is greater benefit from neutralizing certain reactive oxygen metabolites than others. However, in contrast to many other potential therapeutic agents, DMTU enters tissues readily and has a long half-life in vivo. These properties (rapid delivery to the site under attack and prolonged effectiveness for at least 24 hours) may be necessary to accomplish meaningful reduction in myocardial necrosis with antioxidants. Agents with these properties, including DMTU, warrant further evaluation for potential clinical application. In general, clinical usefulness probably requires that an agent be capable of reducing injury with administration after ischemia is well established.

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