Oxygen radicals trigger activation of NF-κB and AP-1 and upregulation of ICAM-1 in reperfused canine heart

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Oxygen radicals trigger activation of NF-κB and AP-1 and upregulation of ICAM-1 in reperfused canine heart. Am J Physiol Heart Circ Physiol 282: H1778–H1786, 2002; 10.1152/ajpheart.00796.2000.—We investigated whether oxygen radicals generated during ischemia-reperfusion trigger postischemic inflammation in the heart. Closed-chest dogs underwent 90-min coronary artery occlusion, followed by 1- or 3-h reperfusion: 10 dogs received the cell-permeant oxygen radical scavenger N-(2-mercaptopropionyl)-glycine (MPG; 8 mg·kg⁻¹·h⁻¹ intracoronary) beginning 5 min before reperfusion, and 9 dogs received vehicle. Blood flow (microspheres), intercellular adhesion molecule (ICAM)-1 protein expression (immunohistochemistry), ICAM-1 gene activation (Northern blotting), nuclear DNA binding activity of nuclear factor (NF)-κB and AP-1 (electrophoretic mobility shift assays), and neutrophil (PMN) accumulation (myeloperoxidase activity) were assessed in myocardial tissue samples. ICAM-1 protein expression was high in vascular endothelium after ischemia-reperfusion but was markedly reduced by MPG. MPG treatment also markedly decreased expression of ICAM-1 mRNA and tissue PMN accumulation. Nuclear DNA binding activities of NF-κB and AP-1, increased by ischemia-reperfusion, were both markedly decreased by MPG at 1 h of reperfusion. However, by 3 h, AP-1 activity was only modestly reduced by MPG and NF-κB activity was not significantly different from ischemic-reperfused controls. These results suggest that oxygen radicals generated in vivo during reperfusion trigger early activation of NF-κB and AP-1, resulting in upregulation of the ICAM-1 gene in vascular endothelium and subsequent tissue accumulation of activated PMNs.

reperfusion injury; oxidative stress; neutrophils; vascular endothelium

RESTORATION OF BLOOD FLOW to ischemic tissue may result in acute inflammation and an extension of ischemia-related tissue damage. The genesis of postischemic inflammation is complex and involves activation of vascular endothelium, genetic upregulation of endothelial cell adhesion proteins and proinflammatory cytokines, and infiltration of neutrophils (PMNs). Intercellular adhesion molecule (ICAM)-1 is thought to play a central role in the trapping and accumulation of activated PMNs in ischemic-reperfused myocardium (21). Monoclonal antibodies directed against ICAM-1 or its ligand on PMNs (the integrins CD11a/CD18 and CD11b/CD18) reduce cardiac microvascular and parenchymal cell injury in animal models (14, 27, 48). In addition, mutant mice deficient in ICAM-1 are less susceptible to cerebral (43) and renal (19) damage after transient ischemia-reperfusion.

On the basis of in vitro models, the ICAM-1 gene is thought to be regulated by the nuclear transcription factor nuclear factor (NF)-κB, which is normally complexed to the cytoplasmic inhibitory protein IκB. Through a cascade of kinase enzymes, including protein kinase C (38), tyrosine kinases (36), and IκB kinases (9) and involving the intracellular generation of reactive oxygen species (ROS) (22), IκB is phosphorylated, ubiquitinated, and degraded by proteasomes, which release NF-κB from IκB and allow it to translocate to the nucleus. Inhibition of NF-κB by diverse means has been shown to block ICAM-1 induction in vitro (26, 35, 38, 46) and in intact hearts (23).

The importance of ROS in these processes is unclear. In vitro, antioxidants can block NF-κB activation in many but not all cell types (3, 22). In addition, the involvement of ROS appears to be strongly stimulus dependent (3). ROS, by changing cellular redox state, may induce or enhance NF-κB activation by modifying the activity of one or more of the kinase enzymes in the NF-κB activation cascade (22). However, ROS could also directly regulate gene transcription independent of NF-κB and could enhance transcription by activating other redox-sensitive transcription factors, including AP-1 (41). A distinct H₂O₂-responsive element has been localized in the ICAM-1 promoter, separate from the NF-κB binding site, containing binding sites for AP-1 and Ets (39).

This study was done to determine whether ROS generated at the time of coronary reperfusion play an important role in vivo in the activation of transcription factors NF-κB and AP-1, the expression of ICAM-1, and the initiation of acute inflammation in postischemic myocardium.

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METHODS

Healthy adult mongrel dogs were anesthetized with thiopental sodium (25 mg/kg iv), intubated, and ventilated with 0–2% halothane, with the concentration adjusted to maintain a stable arterial pressure. Through small skin incisions, 8-Fr sheaths were placed in both femoral arteries. A 6-Fr pigtail catheter was passed through the left sheath into the left ventricle for injection of microspheres to measure blood flow. The sidearm was used for blood pressure monitoring and microsphere sampling. A 7-Fr guiding catheter was introduced through the right sheath and advanced to the aortic root.

After stabilization, an angioplasty catheter (balloon 10 mm in length and 2.5–3.5 mm in diameter) was inserted through the guiding catheter into the proximal left anterior descending coronary artery (LAD). Myocardial ischemia was induced by inflating the balloon to 3–5 atm to occlude the LAD. After 90 min, the balloon was completely deflated and the myocardium was reperfused. Additional dogs (n = 5) had all of the instrumentation described above but without balloon inflation (sham occlusion). Coronary angiography was performed before and shortly after coronary occlusion, just before balloon deflation, and at the end of reperfusion to confirm that complete arterial occlusion and reperfusion were achieved.

Dogs were randomly assigned to two groups. In the first group (I/R-MPG; n = 7), intracoronary infusion of N-(2-mercaptopropionyl)-glycine (MPG, 8 mg·kg⁻¹·h⁻¹; Sigma) began 5 min before reperfusion and ended just before death 3 h later. The infusion was administered through the central lumen of the balloon catheter into the distal coronary artery with a volumetric infusion pump (IMED, San Diego, CA). MPG was dissolved in a mixture of 77% heparinized normal saline and 23% water (isosmotic with plasma) to a concentration of 10 mg/ml. In the second group (I/R; n = 6), dogs received an equivalent volume of vehicle for 3 h, beginning 5 min before reperfusion. An identical protocol was carried out in another series of dogs, except that reperfusion and intracoronary infusion of MPG or vehicle was carried out for only 1 h (n = 3 each for MPG and vehicle). These latter animals were used for assessment of NF-κB and AP-1 activation based on our finding that activation of these transcription factors peaks at 1 h of reperfusion in this model.

At the end of the reperfusion period, hearts were arrested by rapid intravenous infusion of potassium chloride and removed from the chest and the left ventricle was opened flat. Tissue specimens (4 g) were quickly excised from the center and the border of the ischemic-reperfused myocardium as well as from the nonischemic region of the left ventricle. A consistent sampling protocol was used to obtain five transmural samples from the ischemic anterior wall and two from the nonischemic posterior wall. Samples were crudely defined as being "central" or "border" within the ischemic region based on the gross anatomic distribution of the LAD. Each sample was divided into three full-thickness pieces, which were then further divided into inner (endocardial) and outer (epicardial) halves. The central portion of each sample was used for microsphere blood flow determination; a second portion was processed for immunohistochemical staining of ICAM-1 protein; the third was fast frozen in liquid nitrogen and stored at −80°C for ICAM-1 mRNA analysis, assessment of NF-κB or AP-1 DNA binding activity, or measurement of myeloperoxidase (MPO) activity (an index of PMN accumulation). In dogs reperfused for 1 h, the frozen samples were used only for assessment of NF-κB or AP-1. In each heart, at least four samples were analyzed for ICAM-1 protein, ICAM-1 mRNA, and NF-κB and AP-1 DNA binding and three samples were analyzed for MPO activity. The need for rapid freezing of myocardial samples precluded measurements of myocardial infarct size.

Regional myocardial blood flow measurement. Regional myocardial blood flow was determined with radioactive (DuPont, North Billerica, MA) or fluorescent nonradioactive (NuFLOW; Interactive Medical Technology, Los Angeles, CA) microspheres at baseline, 80 min after occlusion, 10 min after reperfusion, and 10 min before death with standard techniques (4, 15).

Immunohistochemistry. The location and extent of ICAM-1 protein expression were assessed by immunohistochemistry. Fresh myocardial samples were imbedded in optimum cutting temperature compound, immediately frozen in isopentane precooled with dry ice, and stored at −80°C. Cryostat sections were cut 5 μm thick and fixed in alumina-filtered acetone. Staining was performed with the avidin-biotin immunoperoxidase technique. Sections were incubated for 60 min with primary antibody (CL18/6) and subsequently for 30 min with biotinylated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). CL18/6 is an anti-canine ICAM-1 IgG1 antibody developed as previously described (42). Negative controls included omission of the primary and/or secondary antibodies.

Distribution of and changes in ICAM-1 protein expression on the endothelium of arterioles, capillaries, and venules and on cardiomyocytes were observed independently by light microscopy by two of the authors without knowledge of sample location or treatment group. After the entire tissue section was examined, the intensity of ICAM-1 staining on the endothelium of arterioles and venules was scored on an eight-point scale as previously described (30). The intensity of staining of capillaries and myocytes was scored on a four-point scale (0 = absent, 1 = weak and patchy or diffuse, 2 = moderate and patchy or diffuse or strong and patchy, 3 = strong and diffuse).

RNA preparation and Northern blot analysis. Myocardial samples stored at −80°C were homogenized with a Polytron tissue homogenizer. Total RNA was isolated by a modification of the guanidinium-phenol-chloroform extraction method. Approximately 20 μg of total RNA per lane was fractionated by 1% agarose-formaldehyde gel electrophoresis and transferred to a nylon membrane (Gene-Screen Plus, NEN DuPont, Boston, MA). RNA was fixed by cross-linking in a UV Stratalinker-1800 (Stratagene, La Jolla, CA). After being prehybridized for 3 h at 65°C in a mixture of 0.5 M phosphate buffer, 1% BSA, 1% SDS, and 10 mM EDTA, the blots were hybridized overnight under the same stringent conditions with a previously described cDNA probe for canine ICAM-1 mRNA (42). The probe was radiolabeled with [α-³²P]dCTP to a specific activity of >1 × 10⁶ cpm by random priming. Blots were washed, exposed to Kodak X-ray films with intensifying screens for an appropriate time at −80°C, and analyzed by densitometry (Imagequant, Molecular Dynamics, Sunnyvale, CA). To control for variability in the loaded quantity of total RNA, all filters were probed with a 24-bp oligonucleotide probe (5’-ACGGTATCTGTATGCTTCTGGAAC) corresponding to 18S rRNA that was end-labeled with a terminal deoxynucleotidyl transferase (Amersham Life Science, Arlington Heights, IL). The 18S rRNA band was used to normalize mRNA for ICAM-1. Positive-control myocardium was obtained from a pentobarbital sodium-anesthetized dog injected with lipopolysaccharide (LPS; 1 mg/kg iv). The animal was killed 5 h later with an overdose of anesthetic, and the heart was removed and samples were excised from the left ventricular free wall, snap-frozen, and processed as described above.
Preparation of nuclear extracts. Myocardial samples were homogenized at 4°C in hypotonic buffer, filtered, allowed to swell in a cold room for 15 min, and centrifuged at 850 g for 15 min at 4°C. After the supernatants were discarded, the nuclear pellets were washed and proteins were recovered by centrifugation (30 min at 20,000 g). The protein concentration of the supernatant was determined with Coomassie blue. Nuclear extracts were stored at −80°C.

Electrophoretic mobility shift assay. Double-stranded oligonucleotides containing consensus-binding sequences for NF-κB (Promega) were used to assay for binding activity in the nuclear extracts. The oligonucleotides were labeled with [α-32P]dATP by fill-in reaction with the Klenow fragment of DNA polymerase I. Nuclear extracts (5 μg) were mixed with 20,000 cpm of the appropriate 32P-labeled oligonucleotides in 10 μl of buffer, and the reaction products were separated on a 4% nondenaturing polyacrylamide gel. Gels were then dried and exposed to X-ray film at −80°C.

Gel supershift assays were used to verify the identity of bands; 1 μg of the appropriate antibody was added (Santa Cruz Biotechnology) after a 20-min preincubation of extract with labeled oligonucleotide. After incubation for 10 min at room temperature, samples were run on 4% nondenaturing gels. Specificity was confirmed by addition of cold probe in 25× excess.

MPO activity. Frozen myocardial samples from the ischemic and normal regions were homogenized under liquid nitrogen. MPO was released by freeze-thawing and assayed with H2O2 and o-dianisidine as previously described (34).

Statistical analysis. All values are given as means ± SE. Hemodynamic parameters and regional myocardial blood flow during ischemia and reperfusion in each group were compared by repeated-measures analysis of variance. Comparisons of ICAM-1 mRNA and protein expression and of nuclear DNA binding of NF-κB and AP-1 among the two groups and the sham dogs were done by analysis of variance with Duncan’s test for multiple comparisons. MPO activities were compared by Student’s t-test.

RESULTS

Hemodynamics and myocardial blood flow. Of the 19 dogs randomized, 3 were excluded because of technical problems (1 with repeated episodes of ventricular fibrillation, 2 with failure of adequate coronary occlusion). In the remaining animals, there were no significant changes in systolic or diastolic arterial blood pressure or heart rate during coronary occlusion or 3-h reperfusion and no significant differences in these variables at any time point between the I/R and I/R-MPG groups. Similarly, there were no significant differences between the two groups in blood flow to the center or border of the ischemic zone, or to the nonischemic region, at any time point. During coronary occlusion, all dogs demonstrated severe ischemia in the central endocardial portion of the ischemic region (flow ≤ 0.07 ml·min⁻¹·g⁻¹).

Immunohistochemistry of ICAM-1 protein. A high level of ICAM-1 protein expression was seen on the endothelium of venules, arterioles, and capillaries in samples from myocardium reperfused for 180 min in the I/R group (Fig. 1A). ICAM-1 expression was markedly reduced at 180 min of reperfusion in dogs given intracoronary MPG shortly before reperfusion (Fig. 1B), to levels similar to those observed in sham-occluded control animals. As shown by a semiquantitative scoring system (30) and blinded reading of tissue samples, MPG resulted in highly significant decreases in endothelial ICAM-1 staining in arterioles, venules, and capillaries (P < 0.002 for each; Fig. 2). No myocyte staining was observed in any of the experimental groups.

ICAM-1 mRNA expression. ICAM-1 mRNA was present at 180 min of reperfusion in myocardium from the ischemic region in I/R dogs but was undetectable in sham-occluded dogs (Fig. 3). Expression of ICAM-1 mRNA at 180 min of reperfusion was stronger in center compared with border samples. MPG treatment resulted in a decrease in ICAM-1 mRNA expression in both areas (Fig. 3). Quantitation of ICAM-1 bands demonstrated a 32% reduction in expression in central samples and a 41% reduction in border samples in the MPG group (P < 0.08 for central samples, P < 0.02 for border samples, and P < 0.003 for all samples combined; Fig. 4).

Activation of nuclear transcription factors. Both NF-κB and AP-1 binding activities were high compared with those of sham-occluded controls in nuclear extracts from myocardium reperfused for 1 or 3 h in the I/R group (Figs. 5 and 6). Supershift assays demonstrated that NF-κB complexes contained p65 and p50 subunits but not p52 or c-Rel (Fig. 5C), whereas AP-1 contained mainly c-Fos and, to a lesser extent, c-Jun (Fig. 6C). Quantitation of bands by densitometry revealed that MPG produced a marked reduction in DNA binding activity for both transcription factors at 1 h of reperfusion (NF-κB, 88% vs. 15% of LPS-positive control; AP-1, 86% vs. 22% of LPS-positive control; both P < 0.001) to levels not significantly different from those in sham-occluded dogs (Fig. 7). At 3 h of reperfusion, the MPG treatment effect was much less pronounced. AP-1 binding activity in the MPG group was mildly reduced compared with that in the I/R group (117% vs. 84% of LPS-positive control; P < 0.05), but NF-κB binding activity was not reduced (52% vs. 44% of LPS-positive control). Both AP-1 and NF-κB binding activities in the MPG group were significantly greater at 3 h than in sham-occluded dogs (Fig. 7).

MPO activity. Tissue MPO activity was increased five- to sevenfold in endocardial and epicardial samples from the center of the ischemic region in control dogs reperfused for 180 min (Fig. 8). MPG treatment resulted in an ~70% decrease in MPO activity in pooled endocardial and epicardial samples from the ischemic region (0.028 ± 0.007 vs. 0.100 ± 0.019 IU/100 mg; P < 0.003). Activity in nonischemic myocardium was low in both groups and not significantly different.

DISCUSSION

Our study showed that administration of MPG, a cell-permeant oxygen radical scavenger, beginning shortly before reperfusion resulted in less activation of the redox-sensitive nuclear transcription factors NF-κB and AP-1, reduced expression of ICAM-1 at both mRNA and protein levels, and decreased PMN...
accumulation in the reperfused myocardium. These results suggest that ROS generated at reperfusion are responsible, at least in part, for initiation of postischemic inflammation in vivo through upregulation of ICAM-1 and possibly other important proinflammatory genes.

Role of ROS in activation of NF-κB and AP-1. Our findings are consistent with the hypothesis that oxidant stress leads to activation and nuclear translocation of NF-κB and AP-1 and that these factors, in turn, promote ICAM-1 gene transcription through interaction with specific binding sites in the promoter region. Separate binding sites have been identified for NF-κB and AP-1 (24, 39), but it is not exactly clear how transcription of ICAM-1 is regulated or how ROS are involved in the process. ROS could function merely to regulate the level of transcription factors, but they could also act directly by modifying the binding of transcription factors to DNA or regulating their transcriptional activities after binding (41). ROS could also affect transcription rates by increasing the steadystate level or the rate of spontaneous oscillations of intracellular calcium concentration (10, 18).

Fig. 1. Immunohistochemical staining of intercellular adhesion molecule (ICAM)-1. A: control dog with 90-min ischemia and 3-h reperfusion (×880) showing positive stain in small (20–25 μm)-diameter vessels. B: dog given intracoronary N-(2-mercaptopropionyl)-glycine (MPG) beginning just before reperfusion (×525). Note marked decrease in red vascular endothelial ICAM-1 staining with MPG treatment.

Fig. 2. Effect of MPG on ICAM-1 protein staining score after ischemia-reperfusion (see METHODS). MPG significantly decreased ICAM-1 protein expression in microvascular endothelium, including arterioles, venules, and capillaries. Staining scores in sham-occluded control dogs (n = 5) were as follows: arterioles = 0, venules = 1.92 ± 0.50, capillaries = 0.
Addition of H\textsubscript{2}O\textsubscript{2} to cultured cells can cause activation of NF-\kappaB and AP-1 (8, 40). Hypoxia-reoxygenation or proinflammatory cytokines such as tumor necrosis factor (TNF)-\alpha also induce NF-\kappaB activation through intracellular generation of ROS by a membrane-bound NADPH oxidase (11, 37). In many studies, addition of antioxidants, such as N-acetyl-cysteine or pyrrolidinedithiocarbamate (PDTC), or inhibitors of NADPH oxidase (such as diphenyleneiodium) block cytokine-induced ROS production, NF-\kappaB activation, and/or adhesion molecule expression in endothelial cells (11, 12, 29, 41). However, Bowie and O'Neill (3) argued that much of the in vitro evidence supporting a central role for ROS in NF-\kappaB activation is specific to a particular stimulus in a particular cell line. They showed that the activation of NF-\kappaB by H\textsubscript{2}O\textsubscript{2} is cell specific and distinct from physiological activators such as TNF-\alpha and interleukin-1, whereas inhibition by antioxidants is also cell- and stimulus specific. In our study, MPG markedly reduced NF-\kappaB and AP-1 activation after 1 h of reperfusion but had only a modest effect on AP-1 and...
no effect on NF-κB after 3 h. This suggests that ROS may initiate early transcription factor activation but that additional factors, e.g., cytokines, may contribute to persistence of early activation or new activation over several hours.

Few in vivo studies have addressed the role of ROS in transcription factor activation and adhesion molecule expression after ischemia-reperfusion. Diethyldithiocarbamate (DDC) was shown to inhibit activation of NF-κB and expression of cytokine and inducible nitric oxide synthase genes in myocardium after brief ischemia (5). After LPS challenge in rats, PDTC inhibited NF-κB activation, induction of ICAM-1, and infiltration of PMNs in the heart, lungs, and liver (25).

Source and species of ROS. The source and species of ROS responsible for these processes have not been clearly defined. ROS generated during reperfusion are
believed to derive mainly from endothelial cells as a by-product of the xanthine oxidase reaction, although a vascular NADPH oxidase containing the small GTP-binding protein Rac-1 may also contribute (44). Tissue macrophages and activated PMNs probably represent a significant external source of ROS during ischemia-reperfusion. Although O$_2^-$ is the initial species of ROS generated from these various sources, a series of chemical reactions result in the formation of H$_2$O$_2$, HO•, lipid peroxides, peroxynitrite, and other species.

MPG, by virtue of being cell permeant, probably exerts its major impact by scavenging ROS formed within the endothelial cells, although ROS generated externally and crossing the endothelial cell membrane would also be scavenged. MPG undoubtably blunts the burst of ROS produced by hypoxia-reoxygenation (2) but could also scavenge those intracellular ROS formed as a result of inflammatory cytokine-induced activation of endothelial cells. MPG appears to have no effect on the PMN NADPH oxidase (7).

MPG is a potent OH scavenger but a much less efficient scavenger of O$_2^-$ (2). Although OH may have been the ROS responsible for NF-$\kappa$B and AP-1 activation, MPG is a thiol compound and may have worked by replenishing reduced sulhydryl groups and reducing oxidative stress. In the presence of glutathione peroxidase, reduced glutathione (GSH) reacts more rapidly with MPG than peroxides, tending to replenish reduced GSH stores. In vitro, GSH depletion in human umbilical vein endothelial cells resulted in increased ICAM-1 expression and PMN adhesion, which could be inhibited by introduction of decoy oligonucleotide sequences for NF-$\kappa$B or AP-1 (20). GSH inhibited phosphorylation of IkB by TNF-$\alpha$ (6), whereas overexpression of $\alpha$-glutamylcysteine synthetase (to raise cellular levels of GSH) blocked NF-$\kappa$B and AP-1 activation induced by TNF-$\alpha$ but not by H$_2$O$_2$ (28). MPG has also been shown to inhibit myoglobin-H$_2$O$_2$-mediated peroxidation reactions, independent of OH formation (33).

MPG was shown previously to inhibit the expression of ICAM-1 and the increased PMN adhesion induced by anoxia-reoxygenation in cultured rat aortic endothelial cells (1). In vivo, MPG was shown to block activation of NF-$\kappa$B in the rabbit heart after ischemia-reperfusion (47). When infused during reperfusion, MPG reduced myocardial infarct size by $\sim$50% in canine models (16, 17, 32) but had only equivocal effects in the rabbit (31). Protection by MPG has been attributed to the prevention of ROS-induced membrane oxidation, but our results suggest that inhibition of inflammation-mediated damage might also be responsible. Effective scavenging of ROS or maintenance of cellular redox state may represent a useful therapeutic approach for limiting inflammation-mediated myocardial reperfusion injury.

**Limitations.** Our study addressed whether ROS may trigger the early activation of NF-$\kappa$B and AP-1 after ischemia-reperfusion, leading to rapid ICAM-1 upregulation. Although MPG markedly inhibited transcription factor activation at 1 h of reperfusion, the effect was considerably less pronounced at 3 h, suggesting that MPG might have delayed but not eliminated upregulation of the inflammatory response. However, we did not measure tissue ROS levels or MPG concentrations, so it is not known whether ROS were suppressed equally well or whether tissue MPG levels remained equally high throughout the reperfusion period. The concentration of MPG infused in this study has been shown to markedly suppress ROS production early after ischemia-reperfusion (2), but its effects on ROS production over several hours are unknown. Mechanisms independent of ROS may be responsible for delayed upregulation of proinflammatory proteins.

This study did not identify the cell types exhibiting activation of transcription factors or upregulation of ICAM-1 mRNA after ischemia-reperfusion. These changes may occur in both endothelial cells and myocytes, but in our study, ICAM-1 protein expression was identified by immunostaining only in endothelial cells. In addition, we showed previously (45) that the p65 subunit of NF-$\kappa$B and the c-Fos subunit of AP-1 are both localized primarily in vascular endothelium. On the basis of these data, we believe that the changes we describe in this study occurred principally in vascular endothelial cells.

This study has not defined the precise mechanism of MPG’s effect. As noted above, MPG is an antioxidant with potent OH scavenging properties but is not a specific scavenger for this radical species. MPG could act by replenishing tissue GSH stores or inhibiting peroxidation reactions. In all likelihood, however, its effects in this study are attributable to its antioxidant properties.

Although antioxidants may prove useful in preventing inflammation-related ischemia-reperfusion injury, more work is needed to define which agents are most effective, how long they should be administered, and whether there are any downsides to their use.

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**REFERENCES**


