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HIF-1 activation attenuates postischemic myocardial injury: role for heme oxygenase-1 in modulating microvascular chemokine generation

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Ockaili, Ramzi, Ramesh Natarajan, Fadi Salloum, Bernard J. Fisher, Drew Jones, Alpha A. Fowler III, and Rakesh C. Kukreja. HIF-1 activation attenuates postischemic myocardial injury: role for heme oxygenase-1 in modulating microvascular chemokine generation. *Am J Physiol Heart Circ Physiol* 289: H542–H548, 2005. First published April 1, 2005; doi:10.1152/ajpheart.00089.2005.—The CXC chemokine IL-8, which promotes adhesion, activation, and transmigration of polymorphonuclear neutrophils (PMN), has been associated with production of tissue injury in reperfused myocardium. Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric peptide that is a key regulator of genes such as heme oxygenase (HO)-1 expressed under hypoxic conditions. We hypothesized that HO-1 plays an important role in regulating proinflammatory mediator production under conditions of ischemia-reperfusion. HIF-1 was activated in the human microvascular endothelial cell line (HMEC-1) with the prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG). DMOG significantly attenuated cytokine-induced IL-8 promoter activity and protein secretion and cytokine-induced PMN migration across human microvascular endothelial cell line HMEC-1 monolayers. In vivo studies in a rabbit model of myocardial ischemia-reperfusion showed that rabbits pretreated with a 20 mg/kg DMOG infusion ($n = 6$) 24 h before study exhibited a 21.58 ± 1.76% infarct size compared with 35.25 ± 2.06% in saline-treated ischemia-reperfusion animals ($n = 6$, change in reduction = 39%; $P < 0.001$). In DMOG-pretreated (20 mg/kg) animals, plasma IL-8 levels at 3 h after onset of reperfusion were 405 ± 40 pg/ml vs. 790 ± 40 pg/ml in saline-treated ischemia-reperfusion animals ($P < 0.001$). DMOG pretreatment reduced myocardial myeloperoxidase activity, expressed as number of PMN per gram of myocardium, to 1.43 ± 0.59 vs. 4.86 ± 1.1 ($P = 0.012$) in saline-treated ischemia-reperfused hearts. Both in vitro and in vivo DMOG-attenuated IL-8 production was associated with robust HO-1 expression. Thus our data show that HIF-1 activation induces substantial HO-1 expression that is associated with attenuated proinflammatory chemokine production by microvascular endothelium in vitro and in vivo.

hypoxia-inducible factor 1; cardiac ischemia-reperfusion; interleukin-8

SIGNIFICANT CLINICAL AND EXPERIMENTAL research has focused on protection of ischemic myocardium. Successful protection strategies are diverse and have included sublethal ischemia and certain pharmacological approaches (23, 27, 35). The biological process now recognized as "preconditioning" enhances endogenous cellular mechanisms within the myocardium and results in protection against postischemic injury. Identifying new pharmacological agents that promote long-lasting protection against ischemia-reperfusion injury is an important goal. In this study, we examine a novel pharmacological strategy for cardiac preconditioning using an inhibitor of prolyl hydroxylase, a key enzyme controlling stabilization, and activation of hypoxia-inducible factor-1 (HIF-1). HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β subunits (38, 39), and its activity is determined by stable expression of the α-subunit (11, 12). HIF-1α subunit activity is negatively regulated in normoxic cells by hydroxylation of proline residues that signal ubiquitination and degradation through the proteasome pathways (9, 10). Both prolyl hydroxylase inhibition and hypoxia stabilize HIF-1α protein, which leads to increased levels of active HIF-1α/β heterodimers in cell nuclei (34). Active HIF-1 binds to DNA at sites represented by the consensus sequence 5′-RCGTG-3′ and participates in essential physiological processes such as energy metabolism, iron homeostasis, vascular remodeling, cell proliferation, and angiogenesis via activation of numerous target genes (30, 31).

IL-8 is a CXC chemokine that mediates adhesion, activation, and migration of blood polymorphonuclear neutrophils (PMN) into sites of inflammation. Human studies underscore the consequences of unregulated IL-8 secretion in the generation of cardiac injury (32). Riesenberg and colleagues (26) showed that greater IL-8 serum levels and PMN reactive oxygen species generation predicted extensive ventricular infarct and complicated clinical courses. Neumann et al. (21) demonstrated significantly increased IL-8 levels in coronary sinus blood compared with aortic root blood in human subjects undergoing angioplasty for acute coronary occlusion, indicating that postischemic hearts generate significant IL-8. Elevated plasma IL-8 serum levels have been shown to predict early recurrence of ischemia, myocardial infarction, and sudden cardiac death after percutaneous coronary interventional procedures (25). Monoclonal antibody against IL-8 produced dramatic attenuation of PMN infiltration and tissue injury in the postischemic heart (4). However, as a therapeutic tool, monoclonal antibody infusion has limited application in human medicine.

In the present study, we investigated the role of HIF-1 in the regulation of chemokine secretion in postischemic microvasculature. We show here for the first time that systemic administration of the potent prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG) before ischemia-reperfusion leads to attenuated serum IL-8 levels and myocardial PMN infiltration in the heart and is associated with a significant reduction in

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myocardial infarct size. The attenuation of tissue injury was associated with robust expression of heme oxygenase (HO)-1, a gene regulated by HIF-1 (13). These results are further supported by in vitro studies using human vascular endothelial cells and suggest that HIF-1 regulation of IL-8 expression occurs through the induction of HO-1.

MATERIALS AND METHODS

Reagents and chemicals. DMOG was obtained from Cayman Chemicals (Ann Arbor, MI). The rabbit IL-8 ELISA kit (OptEIA Set) was obtained from BD Pharmingen (San Diego, CA). The human-specific IL-8 ELISA kit (Quantikine) was obtained from R&D Systems (Minneapolis, MN). Ketamine anesthetic (Ketalar, Parke-Davis) was obtained from Sigma (St. Louis, MO). RNeasy RNA isolation kits and Effectene transfection reagents were obtained from Qiagen (Valencia, CA). Hypoxia chambers (Modular Incubator Chamber) were obtained from Billups-Rothenberg (Del Mar, CA). Immobilon membranes were obtained from Millipore (Bedford, MA). The kit for determining cell viability (Live/Dead) and calcein AM were obtained from Molecular Probes (Eugene, OR). All other chemicals and reagents were obtained from Sigma. The IL-8 promoter vector pGL3-1009-luc was constructed by cloning the proximal 1,009 bp of the human IL-8 promoter into the luciferase promoter vector pGL3-basic (Promega).

Endothelial cell culture. The human microvascular endothelial cell line HMEC-1, obtained from the Centers for Disease Control and Prevention (CDC, Atlanta, GA), was immortalized by Dr. Edwin Ades, Fransisco J. Candal of the CDC, and Dr. Thomas Lawly of Emory University (Atlanta, GA) and was designated HMEC-1. HMEC-1 were cultured as described previously (19).

HO-1 and HIF-1α Western blot analysis. Whole cell and nuclear extracts were isolated from rabbit hearts and HMEC-1 cells as described previously (20, 43). Proteins were resolved by SDS-polyacrylamide gel electrophoresis (4–20%) and electrophoretically transferred to polyvinylidene difluoride membranes (0.45-μm pore size). Immunodetection was performed as follows: immunodection of HO-1 in rabbit hearts with a monoclonal HO-1 antibody (OSA-111, Stressgen), immunodection of HO-1 in HMEC-1 cells with a polyclonal HO-1 antibody (SPA-896, Stressgen), and immunodection of HIF-1α in HMEC-1 cells with a polyclonal HIF-1α antibody (Santa Cruz Biotechnology) and Renaissance Western Blot Chemiluminescence Reagent Plus (PerkinElmer Life Sciences). All membranes were stained with Ponceau S solution (0.2% wt/vol in 1% acetic acid; Sigma) to ensure equal loading and transfer of proteins (17).

Measurement of bilirubin. HO-derived bilirubin was measured in cell culture medium by using a modification of a method described recently (37). Briefly, after treatment of HMEC-1 cells, 0.5 ml of culture supernatant was collected and 250 mg of $BaCl_2 \cdot 2H_2O$ were added. After the sample was vortexed (10–15 s), 0.75 ml of benzene was added and tubes were vortexed again vigorously for 60 s, leading to the formation of a relatively stable milky white emulsion. The benzene phase containing the extracted bilirubin was separated from the aqueous phase by centrifugation at 13,000 *g* for 30 min. Bilirubin was measured spectrophotometrically as a difference in absorbance between 450 and 600 nm (ϵ 27.3 $mM^{-1} \cdot cm^{-1}$), and the amount of bilirubin excreted into the culture medium was expressed as micromolars per milligram of protein. In a separate tube, 0.5 ml of fresh culture medium was processed in the same way, and the benzene layer was collected and used as a blank.

Transient transfections and dual luciferase reporter assay. HMEC-1 were transfected with a hypoxia-response element luciferase reporter vector, pEpo3′Glut1-Luc, which contains a trimer of murine Epo 3′ enhancer and the Glut-1 promoter, or pGL3-1009-luc, which contains the proximal 1,009 bp of the human IL-8 promoter. HMEC-1 cultures were cotransfected with vector pHRL-null (Promega) containing a synthetic *Renilla* gene sequence (hRluc) to enable accurate control for transfection efficiency and indexing of luciferase activity. Cells were transfected with Effectene optimized according to the manufacturer's instructions. Twenty-four hours after transfection, HMEC-1 were incubated under normoxic conditions or exposed to hypoxia (1% O_2) followed by reoxygenation (21% O_2) for different time periods. Additionally, 24 h after transfection, separate cultures were exposed to DMOG (500 μM) or to $CoCl_2$ (150 μM) and cultures were incubated for 4 h to chemically stabilize HIF-1α. Dual luciferase output (Dual-Luciferase Reporter Assay System, Promega) was quantified by luminometer, and results were expressed as an index of relative light units (19).

PMN transendothelial migration. HMEC-1 cells were cultured to confluence on fibronectin-coated (2 $\mu g/cm^2$) polycarbonate filters (Transwell no. 3415, 6.5-mm diameter, 3-μm pore size, 0.33 cm^2). Monolayers were exposed to TNF-α (10 ng/ml) for 4 h and then washed with PBS. Human PMN were isolated via density gradient centrifugation and calcein AM labeled. Labeled PMN were added to the upper wells, followed by incubation (4 h, 37°C). Migration from the upper to lower chamber was assessed by quantifying the numbers of labeled PMN migrating to the lower wells with fluorescence microscopy imaging and an ImagePro software counting function (7). Results are expressed as percentages of total PMN migrated.

Myocardial infarction protocol. The myocardial infarction protocol used in this study has been described previously (22). Briefly, New Zealand White rabbits were anesthetized by an intramuscular injection of ketamine HCl (35 mg/kg) and xylazine (5 mg/kg). Myocardial ischemia was induced by occlusion of the left coronary artery for 30 min, followed by reperfusion for 180 min. All animal experiments were conducted under protocols approved by Virginia Commonwealth University using guidelines on humane use and care of laboratory animals for biomedical research by the National Institutes of Health (Pub. No. 85-23, Revised 1996). The areas of infarction, the risk zone, and the whole left ventricle were measured by computer morphometry with Bioquant imaging software (BIO98) as described previously (22). Infarct size was expressed as a percentage of the ischemic risk area.

IL-8 protein quantification. Species-specific IL-8 chemokine levels in rabbit plasma and cell culture medium from HMEC-1 were quantified on triplicate diluted (5×, 25×, 125×, and 625×) samples by sandwich ELISA according to the manufacturers' directions (BD Pharmingen, R&D Systems).

Tissue myeloperoxidase activity. Six hundred micrograms of tissue samples were obtained from the base of the left ventricle and homogenized (20% wt/vol) in 50 mM phosphate buffer (pH 6) containing 0.5% hexadecyltrimethylammonium bromide for ~30 s at 5,000 rpm. Samples were then centrifuged at 40,000 *g* for 30 min at 4°C. Pellets were resuspended and sonicated three times for 30 s at 4°C. Sonicates were heated for 2 h at 60°C and centrifuged (2,000 *g*, 10 min) at 22°C. Supernatants were analyzed for myeloperoxidase (MPO) activity. Change in absorbance due to the MPO-dependent reaction of *o*-dianisidine dihydrochloride and H_2O_2 was measured at 450 nm over 3 min. Standard curves were prepared from rabbit PMN lysates (0.3–3 × 10^6 PMN/ml), and MPO content was expressed as PMN equivalents per gram of cardiac tissue. Rabbit PMN were isolated as described previously (7).

Statistical analysis. All measurements of infarct size and risk areas are expressed as group means ± SE. Changes in hemodynamics and infarct size variables were analyzed by two-way repeated-measures ANOVA to determine the main effect of time, group, and time-by-group interaction. If the global tests showed major interactions, post hoc contrasts between different time points within the same group or between different groups were performed by *t*-test. Statistical differences were considered significant if the *P* value was <0.05.

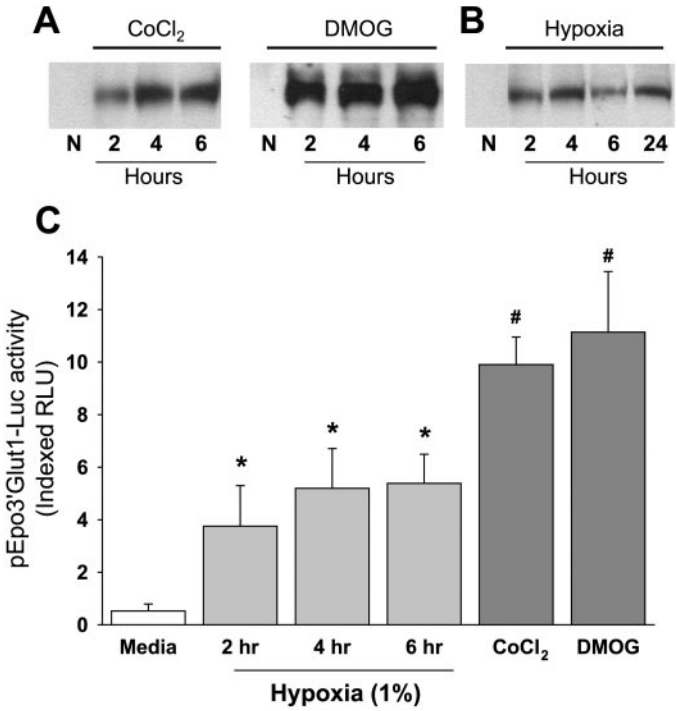

Fig. 1. *A* and *B*: 150 μM $CoCl_2$ and 500 μM dimethyloxalylglycine (DMOG) for 2, 4, and 6 h under normoxic (N) conditions and hypoxia for 2, 4, 6, and 24 h produced strong stabilization of hypoxia-inducible factor (HIF)-1α in HMEC-1 cultures. *C*: 150 μM $CoCl_2$ and 500 μM DMOG for 4 h under normoxic conditions and hypoxia for 2, 4, and 6 h produced strong functional activation of HIF-1 in HMEC-1 cultures. RLU, relative light units. $*P < 0.05$ vs. media (M); $\#P < 0.001$ vs. media.

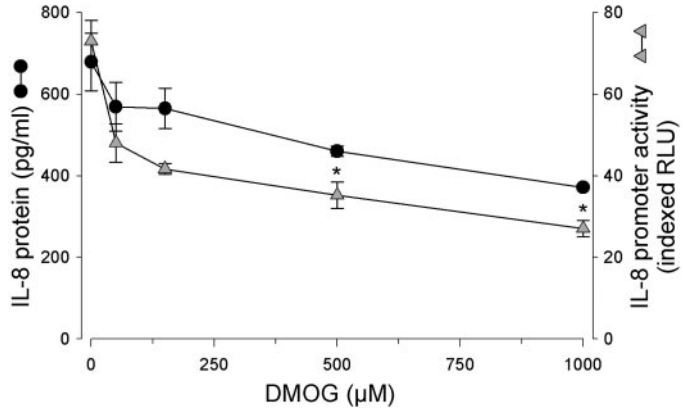

Fig. 2. DMOG produced a concentration-dependent reduction in cytokine-stimulated IL-8 secretion and promoter activity. DMOG concentrations of 500 and 1,000 μM significantly reduced ($*P < 0.01$) TNF-α-stimulated IL-8 promoter activity and protein secretion compared with cells exposed to TNF-α alone.

RESULTS

DMOG promotes HIF-1 activation in HMEC-1 cells. Separate cultures of confluent HMEC-1 were exposed to $CoCl_2$ (150 μM) or DMOG (500 μM) for 2, 4, and 6 h under normoxic conditions. Confluent HMEC-1 were also exposed to hypoxia (1% O_2) for 2, 4, 6, and 24 h. As shown in Fig. 1, *A* and *B*, robust HIF-1α stabilization was observed under normoxic conditions after exposure to $CoCl_2$ and DMOG. Subconfluent HMEC-1 cotransfected with HIF-1α reporter vector pEpo3′Glut1-Luc and pHRL-null were exposed to hypoxia for 2, 4, or 6 h or to 150 μM $CoCl_2$ or 500 μM DMOG for 4 h under normoxic conditions. Figure 1*C* shows that classic stimuli (hypoxia and $CoCl_2$) promoted functional HIF-1 activation in HMEC-1. Prolyl hydroxylase inhibition after DMOG exposure also promoted strong, functional HIF-1 activation.

HIF-1 activation attenuates cytokine-induced IL-8 promoter activity and IL-8 protein secretion in HMEC-1. HMEC-1 cultures were cotransfected with the IL-8 promoter construct pGL3-1009-luc and pHRL-null for 24 h, washed, and exposed to increasing concentrations of DMOG (50–1,000 μM) for 4 h. Subsequently, they were challenged with TNF-α (1 ng/ml). Conditioned medium was harvested 4 h later, and IL-8 protein was quantified by human-specific ELISA. Cells were lysed and analyzed for dual luciferase activity. Figure 2 shows that exposure of HMEC-1 to medium plus TNF-α in the absence of DMOG promoted significant upregulation of IL-8 promoter activity and IL-8 secretion. However, activation of HIF-1 in HMEC-1 via DMOG exposure produced a concentration-dependent reduction in cytokine-stimulated activity of the promoter and secretion of IL-8. DMOG concentrations of 500 and 1,000 μM significantly reduced ($P < 0.01$) TNF-α-stimulated IL-8 promoter activity and protein secretion compared with control cells exposed to TNF-α alone.

HIF-1 activation attenuates cytokine-induced PMN transmigration in HMEC-1. We next examined the impact of HIF-1 activation on a key endothelial cell function in part mediated by IL-8. HMEC-1 were cultured to confluence on polycarbonate membranes in transwell chambers. Triplicate monolayer cultures were exposed to medium alone, medium plus TNF-α (1 ng/ml), or medium plus TNF-α (1 ng/ml) after a 4-h 500 μM DMOG exposure. Monolayers were then washed three times in PBS, and calcein AM-labeled human PMN (1×10^6/ml) were added to the upper chambers. The transwells were then incubated (4 h, 37°C). Migration from the upper to the lower chamber across HMEC-1 monolayers was assessed as described above. Figure 3 shows that TNF-α-activated HMEC-1 monolayers promote significant increases in PMN migration

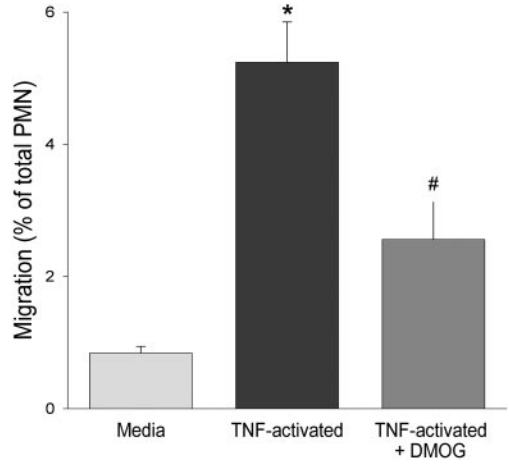

Fig. 3. TNF-α induced significant increases in polymorphonuclear neutrophil (PMN) migration across human microvascular endothelial cell line (HMEC-1) monolayers compared with HMEC-1 exposed to medium alone ($*P < 0.001$). Pretreatment with DMOG before TNF-α activation produced significant reduction in PMN migration compared with TNF-α alone ($\#P < 0.01$).

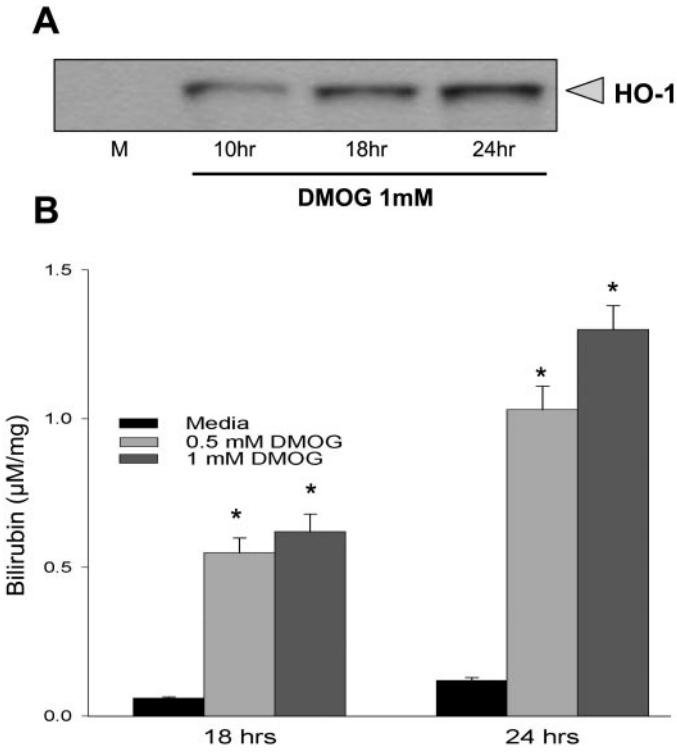

Fig. 4. Effect of DMOG on heme oxygenase (HO)-1 expression and activity by HMEC-1. *A*: 1 mM DMOG produced a time-dependent increase in HO-1 protein over a 24-h period. *B*: both 0.5 and 1.0 mM DMOG concentrations produced significant increases (*$P < 0.001$) in HO-1 activity after 18 and 24 h of incubation. Activity was assessed by bilirubin production.

compared with medium alone ($P < 0.001$). Exposing monolayers to DMOG before TNF-α activation reduced PMN migration compared with TNF-α alone ($P < 0.01$), suggesting that HIF-1 activation attenuates cytokine-induced proinflammatory events in microvascular endothelium.

HIF-1 activation promotes HO-1 expression in HMEC-1. Emerging research suggests that HIF-1 activation in a number of cell systems promotes HO-1 expression. We examined the extent to which activation of HIF-1 via DMOG exposure induces HO-1 expression and activity in microvascular endothelium. HMEC-1 were exposed to medium alone or to medium containing DMOG at 0.5 and 1.0 mM and incubated for 10, 18, and 24 h. After exposure, conditioned medium and cell lysates were collected. HO-1 activity was quantified by bilirubin determination as described above. Whole cell extracts were isolated, and Western blot analysis was performed for HO-1. As shown in Fig. 4*A*, HIF-1 activation after exposure to 1 mM DMOG for 10, 18, and 24 h induced a time-dependent increase in HO-1 expression. When HMEC-1 were exposed to 0.5 and 1.0 mM DMOG for 18 and 24 h, we observed significantly increased HO-1 activity compared with HMEC-1 incubated in medium alone ($P < 0.01$).

DMOG reduces infarct size after ischemia-reperfusion injury. Two groups of rabbits were studied: *1*) saline-infused ischemia-reperfusion injured animals received sterile saline administrated intraperitoneally 24 h before cardiac ischemia-reperfusion injury; and *2*) DMOG-treated ischemia-reperfusion injured animals received sterile saline plus 20 mg/kg DMOG administered intraperitoneally 24 h before cardiac ischemia-reperfusion injury. Table 1 shows hemodynamic data in the two study groups. Our results show significantly higher mean arterial blood pressure (MAP) and rate-pressure product (RPP) ($P < 0.05$) in DMOG-treated animals at the conclusion of 30 min of ischemia compared with saline control animals. After 180 min of reperfusion, DMOG-treated hearts showed significantly greater MAP and RPP compared with saline control animals. Figure 5*A* shows the ischemic area of risk between saline-treated control animals and DMOG-pretreated animals. No significant differences were noted between the groups. Figure 5*B* shows that rabbits subjected to ischemia-reperfusion injury alone ($n = 6$) exhibited a 35.25 ± 2.06% infarct size. Rabbits treated with DMOG for 24 h ($n = 6$) before ischemia-reperfusion exhibited reduction in infarct size to 21.58 ± 1.76% (change in reduction = 39%, $P < 0.0001$).

DMOG attenuates postischemic plasma IL-8 generation and PMN sequestration in myocardium. Plasma specimens from all animals were analyzed at 30-min intervals for IL-8 by rabbit-specific IL-8 ELISA. Figure 6 shows that control animals receiving only saline exhibited surges in plasma IL-8 within 2 h after onset of reperfusion compared with baseline, preischemia IL-8 values ($P < 0.001$). DMOG attenuated plasma IL-8 levels compared with saline-treated ischemia-reperfusion control animals at 240 min ($P < 0.001$). Figure 7 shows that hearts obtained from saline-treated ischemia-reperfused animals exhibited significant increases in myocardial MPO content compared with nonischemic control animals ($P < 0.001$). DMOG-treated hearts showed attenuated myocardial MPO activity compared with saline-treated ischemia-reperfusion hearts ($P = 0.012$).

Table 1. *Hemodynamic data*

				Reperfusion		
	Baseline	Preischemia	30-min Ischemia	60 min	120 min	180 min
Control group						
HR	199±8	213±9	209±9	191±10	182±10	179±10
MAP	94±3	84±4†	79±4*†	73±3*†	72±3†	67±2*†‡
RPP	21,072±950*	21,114±842	19,154±781*	16,517±855†‡§	14,871±978†‡§	13,519±928*†‡§
DMOG-treated group						
HR	216±5	210±2	216±18	205±14	187±9	183±9
MAP	98±2	95±3	96±6	88±4	83±6†	81±5†
RPP	24,859±1,239	22,848±2,027	23,248±1,617	21,647±2,274	17,738±860†	17,965±1,336†

Values are means ± SE. DMOG, dimethyloxalylglycine; HR, heart rate (beats/min); MAP, mean arterial blood pressure (mmHg); RPP, rate-pressure product (mmHg·beats·min^{-1}). *$P < 0.05$ vs. DMOG; †$P < 0.05$ vs. baseline; ‡$P < 0.05$ vs. preischemia; §$P < 0.05$ vs. 30-min ischemia.

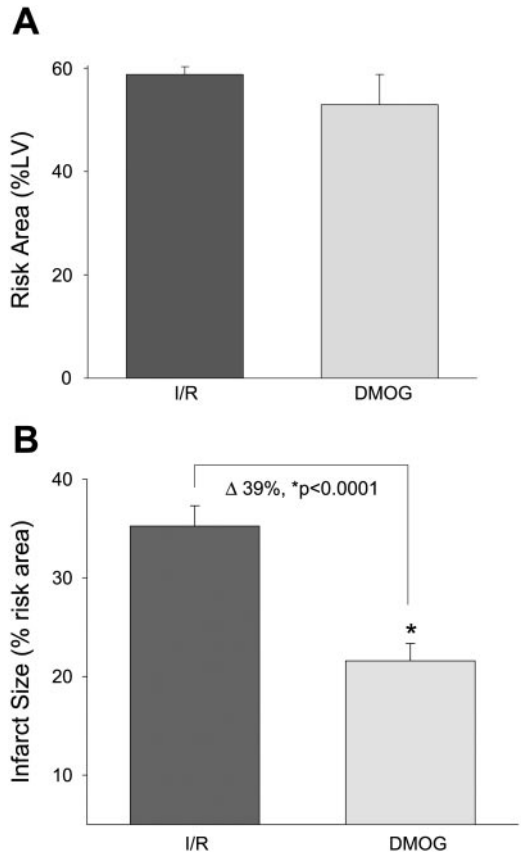

Fig. 5. *A*: at risk areas in saline-treated ischemia-reperfused (I/R) animals and DMOG-pretreated animals. No significant differences were noted. LV, left ventricle. *B*: effect of DMOG (20 mg/kg) pretreatment ($n = 6$) on infarct size in postischemic rabbit hearts compared with saline-treated I/R control hearts ($n = 6$). DMOG pretreatment produced a significant reduction ($*P < 0.001$) in myocardial infarct size compared with saline-treated control hearts.

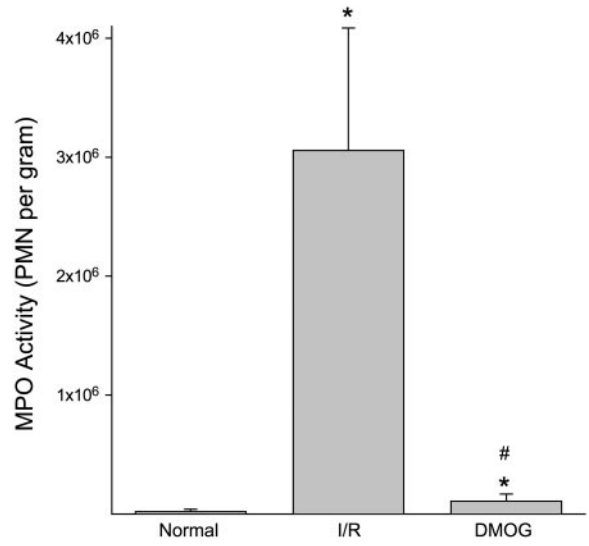

Fig. 7. Effect of DMOG pretreatment ($n = 6$) on myocardial myeloperoxidase (MPO) activity in postischemic rabbit hearts compared with saline-treated I/R control hearts ($n = 6$). MPO activity in saline-treated control hearts was significantly increased ($*P < 0.001$) compared with normal hearts not subjected to I/R injury ($n = 3$). DMOG pretreatment significantly reduced ($\#P < 0.001$) MPO activity compared with saline-treated I/R hearts.

DMOG promotes HO-1 expression in postischemic myocardium. Tissue extracts from saline-treated hearts exposed to ischemia-reperfusion injury showed a modest induction of HO-1 expression compared with nonischemic control hearts (Fig. 8). DMOG-treated ischemia-reperfusion hearts showed increased HO-1 expression compared with saline-treated hearts after ischemia-reperfusion ($P < 0.05$). Furthermore, DMOG induced substantial HO-1 expression in saline-treated control hearts (data not shown).

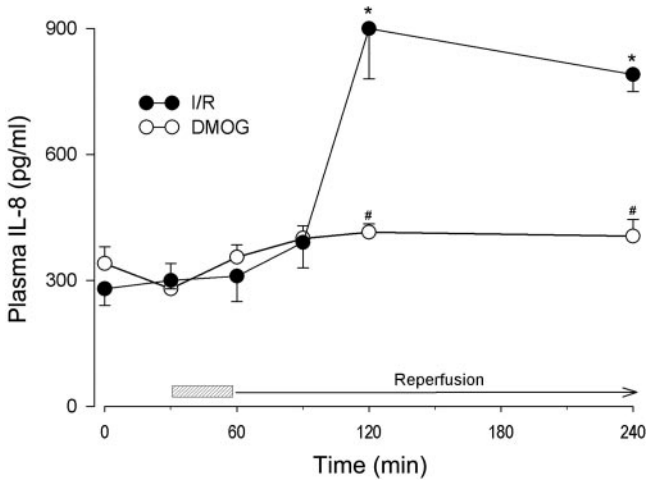

Fig. 6. Effect of DMOG pretreatment ($n = 6$) on plasma IL-8 levels in postischemic rabbit hearts compared with saline-treated I/R control hearts ($n = 6$). Saline-treated I/R control animals exhibited significant ($*P < 0.001$) increases in circulating IL-8 at 180 and 240 min compared with baseline (0 min). Surges in circulating IL-8 were abolished by DMOG preconditioning. Plasma IL-8 levels in DMOG-pretreated animals were significantly lower than in saline-treated control animals at 180 and 240 min ($\#P < 0.001$).

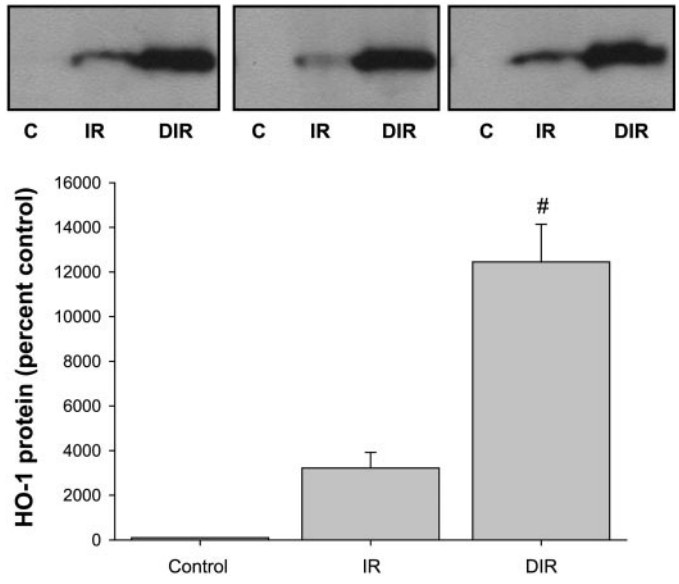

Fig. 8. Effect of DMOG preconditioning on cardiac HO-1 expression. Cell extracts were isolated from control nonischemic hearts (C, $n = 3$), saline-treated I/R hearts ($n = 3$), and DMOG-pretreated hearts (DIR, $n = 3$). The normal hearts exhibited virtually no detectable HO-1. HO-1 signal intensity increased in saline-treated I/R hearts. DMOG pretreatment significantly increased HO-1 expression compared with saline-treated I/R hearts ($\#P < 0.05$).

DISCUSSION

Transient sublethal ischemia preconditions myocardium to withstand subsequent sustained ischemia and reperfusion. Ischemic preconditioning substantially reduces infarct size under these conditions (5) and induces robust resistance to infarction for early periods of up to 2 h (14). A second, less protective period appears 12–24 h later and persists for ~72 h (3). Emerging research implicates roles for endogenous opioids (28), norepinephrine (2), reactive oxygen and nitrogen species (36, 42), and adenosine (15) in ischemic preconditioning. Hearts preconditioned by "chemical stimuli" exhibit similar resistance to ischemia and reperfusion. Chemical agents such as nontoxic derivatives of endotoxin monophosphoryl lipid A, openers of ATP-sensitive potassium channels, inhibitors of oxidative phosphorylation, and sildenafil produce both early and delayed cardiac preconditioning to ischemic stress (22, 23, 40). In the present study, we show that a prolyl hydroxylase inhibitor that is known to induce functional activation of HIF-1 significantly reduced infarct size after ischemia-reperfusion injury 24 h later.

In mammalian cell systems, we (19) and others have shown that IL-8 generated by microvascular endothelium during ischemia-reperfusion coordinates PMN adhesion and transvascular migration. A gathering body of work from human studies underscores the consequences of IL-8 generation in development of devastating organ injury (21, 25, 26). Clinical studies in humans and related studies in animal model systems suggest that attenuation of IL-8 activity reduces vascular injury (4). However, the primary tool to downregulate the biological activity of IL-8 has required IL-8 monoclonal antibody infusion. Dramatic attenuation of PMN infiltration and tissue injury has been achieved in the postischemic lung, heart, and brain with this approach (4, 18, 29). As a therapeutic tool, monoclonal antibody infusion has limited application in human medicine. Therefore, enhancing knowledge of mechanisms that attenuate IL-8 expression is essential for designing new strategies for limiting tissue injury after ischemia-reperfusion. In the present study, we show that DMOG reduced plasma IL-8 levels and myocardial PMN infiltration in postischemic rabbit hearts (Figs. 6 and 7).

Although a direct role for HIF-1 in regulating postischemic inflammation remains uncertain, HIF-1 has been shown to regulate enzyme systems that modulate inflammation. HIF-1 upregulates both HO-1 (13) and nitric oxide (NO) synthase (NOS) (16, 6). CO and NO, produced by induction of HO-1 and NOS, respectively, exert downstream effects on enzyme systems known to regulate inflammation (e.g., soluble guanylate cyclase, MAPK signaling pathways) (1). The mechanism(s) by which CO and NO modulate inflammation remains incompletely defined. In the present study, we observed significant induction of HO-1 expression in postischemic rabbit hearts after infusion of DMOG (Fig. 8). We correlated these observations with in vitro studies showing that HO-1 expression and activity was significantly induced by DMOG in microvascular endothelium (Fig. 4). Inducible HO-1 metabolizes heme to free iron, the antioxidant biliverdin, and CO. Thus HO-1 induction subjects target tissues to increased CO levels. Several studies point to a critical cytoprotective and anti-inflammatory role for HO-1 activity through overlapping pathways. Yet and colleagues (41) found significant reductions in postischemic infarct size in cardiac-specific transgenic mice overexpressing HO-1. The water-soluble CO-releasing molecule tricarbonylchlororuthenium(II) given at the onset of cardiac reperfusion in wild-type mice reduced myocardial infarct size (8, 33). Otterbein and colleagues (24) used dominant-negative mutants and mice deficient in genes for certain MAPKs and demonstrated that CO exerts cytoprotective effects by activation of the MKK3/p38 MAPK pathway. In the current study, DMOG pretreatment resulted in robust HO-1 expression both in vitro and in vivo. Furthermore, identical concentrations of DMOG pretreatment that induced in vitro HO-1 expression were associated with a progressive reduction in cytokine-stimulated IL-8 promoter activity and IL-8 secretion (Fig. 2). Diminished IL-8 secretion after DMOG treatment also resulted in highly significant reductions in cytokine-induced PMN transmigration across microvascular endothelial cell monolayers (Fig. 3). In vivo, DMOG preconditioning abolished IL-8 generated by postischemic hearts (Fig. 6), which in turn abolished myocardial PMN sequestration (Fig. 7). Thus our results point to a novel anti-inflammatory role for HIF-1 in the setting of ischemia-reperfusion injury.

In conclusion, we have demonstrated for the first time that prolyl hydroxylase inhibition induces a delayed preconditioning-like protection effect in the heart after ischemia-reperfusion injury. In addition, we observed attenuation of IL-8 and a reduction in PMN sequestration in postischemic hearts treated with DMOG. Furthermore, our results show that DMOG promoted significant HO-1 expression in postischemic rabbit hearts. Additional investigations are needed to explore this novel mechanism of preconditioning involving HIF-1 activation and HO-1 expression. Advancing knowledge of fundamental mechanisms that repress IL-8 secretion will help devise molecular targets for intervention. Given the prevalence of ischemia-reperfusion injury in humans, the outcome of this research will have important therapeutic relevance.

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