Inhibition of matrix metalloproteinase activity in vivo protects against vascular hyporeactivity in endotoxemia

Jonathan J. Cena,1 Manoj M. Lalu,1 Woo Jung Cho,1 Ava K. Chow,2 Mariel L. Bagdan,1 Edwin E. Daniel,1 Michele M. Castro,1 and Richard Schulz1,2

Departments of 1Pharmacology and 2Pediatrics, Cardiovascular Research Group, University of Alberta, Edmonton, Alberta, Canada

Submitted 20 March 2009; accepted in final form 13 October 2009

Cena JJ, Lalu MM, Cho WJ, Chow AK, Bagdan ML, Daniel EE, Castro MM, Schulz R. Inhibition of matrix metalloproteinase activity in vivo protects against vascular hyporeactivity in endotoxemia. Am J Physiol Heart Circ Physiol 298: H45–H51, 2010. First published October 16, 2009; doi:10.1152/ajpheart.00273.2009.—Persistent arterial hypotension is a hallmark of sepsis and is believed to be caused, at least in part, by excess nitric oxide (NO). NO can combine with superoxide to produce peroxynitrite, which activates matrix metalloproteinases (MMPs). Whether MMP inhibition in vivo protects against vascular hyporeactivity induced by endotoxemia is unknown. Male Sprague-Dawley rats were administered either bacterial lipopolysaccharide (LPS, 4 mg/kg ip) or vehicle (pyrogen-free water). Later (30 min), animals received the MMP inhibitor doxycycline (4 mg/kg ip) or vehicle (pyrogen-free water). After LPS injection (6 h), animals were killed, and aortas were excised. Aortic rings were mounted in organ baths, and contractile responses to phenylephrine or KCl were measured. Aortas and plasma were examined for MMP activity by gelatin zymography. Aortic MMP and inducible nitric oxide synthase (iNOS) were examined by immunoblot and/or immunohistochemistry. Doxycycline prevents the LPS-induced development of ex vivo vascular hyporeactivity to phenylephrine and KCl. iNOS protein was significantly upregulated in aortic homogenates from endotoxemic rats; doxycycline did not alter its level. MMP-9 activity was undetectable in aortic homogenates from LPS-treated rats but significantly upregulated in the plasma; this was attenuated by doxycycline. Plasma MMP-2 activities were unchanged by LPS. Specific MMP-2 activity was increased in aortas from LPS-treated rats. This study demonstrates the in vivo protective effect of the MMP inhibitor doxycycline against the development of vascular hyporeactivity in endotoxemic rats.

SEPSIS REMAINS THE LEADING cause of death in North American intensive care units (20). It is characterized by both an infection and a systemic inflammatory response. Because the cardiovascular symptoms of sepsis dominate its clinical presentation, it is important to understand the mechanisms involved in its cardiovascular pathophysiology. Important symptoms include intrinsic myocardial dysfunction and a marked persistent arterial vasodilation. The development of persistent arterial vasodilation involves the emergence of two interacting factors: overproduction of endogenous vasodilatory substances and vascular hyporeactivity to vasoconstrictors (8). Vasodilators overproduced in septic shock include both nitric oxide (NO) and prostaglandins. Vascular hyporeactivity to vasoconstrictors is characterized by the reduced sensitivity of the vasculature to vasopressor agents. A large body of evidence supports the pathophysiological generation of excess NO in septic shock through the expression of inducible NO synthase (iNOS) in the vasculature and myocardium, which contributes to vasodilatation, vascular hyporeactivity, and cardiac collapse (2, 10, 15, 16, 32, 35, 38, 45, 49).

NO combines with superoxide to form peroxynitrite, a highly reactive species involved in lipid peroxidation, protein and DNA damage, and ion channel and transporter malfunction (32, 44). Its biosynthesis is enhanced in aortas from lipopolysaccharide (LPS)-treated rats (45), thus resulting in endothelial dysfunction (43). Peroxynitrite activates matrix metalloproteinases (MMPs), including MMP-2 (30, 47), a family of zinc-dependent endopeptidases that are involved in a number of physiological and pathological processes and several cardiovascular pathologies (7, 11, 40). Peroxynitrite directly activates the zymogen form of MMPs without requiring the proteolytic removal of the propeptide domain (30, 47); the latter form of activation is important in the canonical extracellular actions of MMPs (24). Because there is increased oxidative stress in the form of peroxynitrite in the heart (16, 19) and vasculature (46) during sepsis, we hypothesized that MMP activity would also be increased. Previous studies have demonstrated that the mRNAs for several MMPs are upregulated in the liver, spleen, and kidney after bacterial LPS administration (33, 34) and that selective deletion of the MMP-9 gene protects against LPS-induced mortality (9).

Tetracyclines, a class of antibiotics that have a bacteriostatic effect on microorganisms, are now recognized to inhibit MMP activity independently of their antibacterial action (13). Of the tetracycline class, doxycycline is the most potent MMP inhibitor and exhibits MMP inhibition in vivo at plasma levels that are subantimicrobial (12). Tetracyclines have been shown to have protective actions via their MMP inhibitory actions in models of cardiovascular injury (25, 40), cancer (14), and inflammation (13). As such, tetracyclines have been examined for their therapeutic potential in models of endotoxic shock (3, 42). Our laboratory has previously shown that doxycycline administration reduced the depression in cardiac contractile function in LPS-treated rats (22). Furthermore, broad spectrum MMP inhibition using a chemically modified tetracycline (which is devoid of antibacterial effects yet retains MMP inhibitory capability) also improved LPS-induced lung injury in a model of acute respiratory distress syndrome in pigs (3). The authors analyzed MMP activity in the bronchoalveolar lavage and injury of lung tissue of LPS-exposed pigs.

Previously, our laboratory studied LPS-induced vascular hyporeactivity in rats which revealed evidence for activation of aortic MMP-2 and the beneficial effects of MMP inhibition in...
vitro in improving vascular hyporeactivity to vasoconstrictors using isolated rat aorta treated with LPS or interleukin-1β in vitro (21). To date, however, no study has examined the effect of MMP inhibition in vivo on changes in vascular hyporeactivity following LPS treatment. We therefore tested the hypothesis that MMPs contribute to the development of vascular hyporeactivity to vasoconstrictors using a model of acute endotoxemia in rats and that inhibition of MMPs with doxycycline would protect against the loss in vascular contractile tone.

**MATERIALS AND METHODS**

All experiments were approved and performed in accordance with the Canadian Council on Animal Care Guide to the Care and Use of Experimental Animals.

**Rat model of endotoxemia.** Male Sprague-Dawley rats (250–350 g) were given either a bolus intraperitoneal injection of a nonlethal dose of LPS (Salmonella typhosa, 4 mg/kg; Sigma) or pyrogen-free water vehicle (control). After this initial injection (30 min), groups of animals were also given either the MMP inhibitor doxycycline (4 mg/kg) or pyrogen-free water vehicle. All animals were then killed humanely by pentobarbital sodium overdose (100 mg/kg ip) at 6 h. Previous investigations by our group and others have revealed that blood pressure is most significantly depressed at this time point (22) and that both cardiac function (16, 23) and vascular reactivity to vasoconstrictors (21) are also significantly depressed.

**Tissue preparation and cryosections.** Aortas were rapidly excised, and connective tissue was trimmed away while in Krebs buffer (in mM: 118 NaCl, 4.75 KCl, 1.19 KH2PO4, 1.19 MgSO4·7 H2O, 2.5 CaCl2, 2 H2O, 11.1 mM glucose, and 25 NaHCO3) bubbled with carbogen (95% O2–5% CO2). Aortas were either cut into 5-mm cylindrical segments (two from one aorta) for organ bath studies (see below) or vascular reactivity to vasoconstrictors (21) are also significantly depressed.

**Assessment of vascular reactivity.** Two aortic rings (5 mm in length) were then mounted between L-shaped tungsten wire hooks in organ baths filled with Krebs buffer at 37°C which was continuously bubbled with 95% O2–5% CO2. Isometric tension was measured using force transducers (Grass FT03) and recorded using AcqKnowledge 3.1 software. A tension of 1 g was applied, and the rings were equilibrated for 60 min while exchanging the Krebs buffer at 15-min intervals. Following equilibration, rings were subjected to increasing concentrations of phenylephrine (PE), and the resulting changes in tension were recorded. Vessels were then washed, and the maximal contractile response to KCl (75 mM) was determined.

**Preparation of aorta homogenates.** Excised aorta were either used for functional experiments (described above) or snap-frozen and crushed at liquid nitrogen temperature and then placed in buffer [50 mM Tris·HCl, 3.1 mM sucrose, 1 mM dithiothreitol, 10 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, 2 μg/ml aprotinin, protease inhibitor cocktail (Sigma), and 0.1% Triton X-100, pH 7.4] at a ratio of 1:4 wt/vol at 4°C, and the supernatant was collected.

**Measurement of MMP activities by gelatin zymography.** Gelatinolytic activities of MMP-2 and -9 were examined as previously described (6). Polyacrylamide gels (8%) copolymerized with gelatin (2 mg/ml, type A from porcine skin; Sigma) were prepared. Samples were obtained from the remaining aortas not used for functional experiments. After homogenization, nonheated samples were diluted with water to load a constant amount of protein per lane (20 μg). A standard was loaded into each gel (conditioned medium from phorbol ester-activated HT-1080 cells; American Type Culture Collection) as an internal standard used to normalize activities between gels. Following 1.5 h of electrophoresis, the gels were washed with 2.5% Triton X-100 for 1 h at room temperature (with three changes of solution) to remove SDS. Gels were then incubated for 16–24 h at 37°C in incubation buffer (50 mM Tris·HCl, 150 mM NaCl, 5 mM CaCl2, and 0.05% NaN3). After incubation, the gels were stained with 0.05% Coomassie Brilliant Blue (G-250; Sigma) in a mixture of methanol-acetic acid-water (2.5:1:6.5 vol/vol) and destained in aqueous 4% methanol-8% acetic acid (vol/vol). Gelatinolytic activities were detected as transparent bands against the dark blue background. Zymograms were digitally scanned, and band intensities were quantified using ImageJ 1.36b (National Institutes of Health).

**Western immunoblotting analysis.** Aorta homogenate (10–20 μg) was loaded onto (8–12%) polyacrylamide gels, electrophoresed at a constant voltage of 150 V under reducing conditions, and then electroblotted onto polyvinylidene difluoride membrane (Bio-Rad) in Towbin buffer (20% vol/vol methanol, 25 mM Tris base, 192 mM glycine, 0.05% wt/vol SDS). Positive standards and/or molecular weight standards (Precision plus protein standard, dual color; Bio-Rad) were also loaded into gels to confirm the identity of proteins to be probed. Polyvinylidene fluoride membranes were permeabilized by soaking in methanol for 1 min. Proteins were transferred onto these membranes at 100 V for 1 h. Following transfer, membranes were blocked in 5% wt/vol skim milk powder in TTBS buffer [0.001% vol/vol Tween 20, 2 M Tris (pH 7.6), and 0.10 M NaCl] for 2 h at room temperature or overnight at 4°C. After blocking, membranes were incubated in 5 ml of 5% wt/vol skim milk powder in TTBS buffer with an anti-mouse MMP-2 antibody (1:1,000 dilution, MAB3308, Chemicon), or an anti-mouse iNOS antibody (1:1,000, N3020/L20, Transduction Laboratories) for 1 h at room temperature or overnight at 4°C. After incubation, membranes were washed with TTBS three times at 5-min intervals. Blots were subsequently incubated with the appropriate horseradish peroxidase-conjugated antibodies (either anti-mouse or anti-rabbit; Transduction Laboratories) in 10 ml of 5% wt/vol skim milk powder in TTBS buffer for 1 h at room temperature. After incubation, membranes were washed with TTBS buffer five times at 5-min intervals. Membranes were visualized using the horseradish peroxidase-luminol chemiluminescence reaction kit (Amersham Pharmacia Biotech).

**Immunolabeling.** The cryosections were dried for 30 min at room temperature followed by two washes with 0.3% Triton X-100 in PBS and one wash with PBS alone. The cryosections were incubated with 10% normal donkey serum (no. 566460; Calbiochem) for 1 h to reduce staining of nonspecific proteins before applying primary antibody. Mouse anti-human MMP-2 IgG1 (1:200, MAB3308; Chemicon International), which does not cross react with human MMP-9, was used as the primary antibody. As a secondary antibody, Cy3-conjugated donkey anti-mouse IgG (1:20, no. 715-165-151; Jackson ImmunoResearch Laboratories) was used. During the incubation with any of the antibodies, 2% vol/vol normal donkey serum was added for stabilization of antibodies and reduction of any background staining. All immunolabeling procedures were performed at room temperature. To determine the specificity of immunolabeling, primary or secondary antibodies were omitted.

**Confocal microscopy.** The immunolabeled cryosections were observed using single photon confocal microscope (LSM 510; Carl Zeiss) and saved by LSM 5 Image (Carl Zeiss). Cy3 (red) was scanned by...
LPS injection causes overt signs of endotoxemia. LPS but not its vehicle caused overt symptoms of endotoxemia in the rats that were clearly apparent 6 h after injection. These included lethargic behaviour, piloerection, and porphyrin secretion from the eyes. Doxycycline treatment, however, did not prevent the development of these symptoms.

Concentration response to PE in aorta from endotoxemic rats. LPS injection caused a significant rightward shift in the EC50 of the contractile response to PE and a reduction in the maximum effect (Fig. 1A and B, and Table 1). Doxycycline administered 30 min after LPS significantly reduced the rightward shift and improved the maximal response in the contractile response to PE. Aortas from control animals administered doxycycline showed no significant alterations in their response to PE (Fig. 1B and Table 1). LPS also caused a decrease in the contractile response to 75 mM KCl (Fig. 1C) that was prevented by doxycycline. Doxycycline administration did not alter the contractile response to KCl in aortas from control rats (Fig. 1C).

Aortic iNOS protein levels. Aortic expression of iNOS protein was very evident in homogenates of aorta harvested 6 h after LPS injection (P < 0.05 vs. control, Fig. 2). However, aortic iNOS was not significantly different in LPS-treated rats administered with doxycycline (Fig. 2). This indicates that the protective effects of doxycycline on the development of vascular hyporeactivity in vivo are either downstream or independent of actions involving iNOS.

Aortic homogenate MMP activities and protein content. Zymographic analysis of homogenized aortic tissue from control rats revealed robust MMP-2 activity at 72 kDa (Fig. 3A). LPS did not change aortic MMP-2 activity significantly from control. There was no additional effect of doxycycline treatment. MMP-9 activity was undetectable by zymography in any aortic homogenates (data not shown). Furthermore, in all groups, 64- and 75-kDa MMP-2 activities were generally near or below the detection limit and nonquantifiable. Western immunoblotting analysis of aortic tissue revealed a trend toward decreased MMP-2 protein in aortas from rats treated with LPS (Fig. 3B). Doxycycline did not significantly alter MMP-2 protein levels after LPS treatment. Analysis of MMP-2 activity expressed per unit protein revealed a significant upregulation of specific MMP-2 activity in aortas from LPS-treated rats that was diminished by doxycycline treatment (Fig. 3C).

Immunohistochemistry of aortic cross sections. Immunohistochemical analysis of aortic cross sections from control aortas revealed an abundant level of MMP-2 distributed throughout the aortic wall in both endothelial and smooth muscle cells (Fig. 4). After LPS administration (6 h), a decrease in MMP-2-associated staining was observed in the aortic wall. Doxycycline did not affect the decrease in MMP-2 protein levels; however, a redistribution of MMP-2 toward the intima was apparent.

Plasma gelatinolytic activities. Plasma from control animals possessed strong MMP-2 activities that appeared at 72 and 75 kDa (Table 2), as previously observed (22). LPS injection did not significantly alter 72- or 75-kDa MMP-2 plasma activities, whether in the absence or presence of doxycycline. In control rats, LPS injection caused a significant rightward shift in the EC50 of the contractile response to PE and a reduction in the maximum effect (Fig. 1A and B, and Table 1). Doxycycline administered 30 min after LPS significantly reduced the rightward shift and improved the maximal response in the contractile response to PE. Aortas from control animals administered doxycycline showed no significant alterations in their response to PE (Fig. 1B and Table 1). LPS also caused a decrease in the contractile response to 75 mM KCl (Fig. 1C) that was prevented by doxycycline. Doxycycline administration did not alter the contractile response to KCl in aortas from control rats (Fig. 1C).
animals, MMP-9 plasma activities were detected at 92 kDa and 135 kDa [the former is full length MMP-9, whereas the latter is a lipocalin-associated form of MMP-9 (16)]. After LPS injection (6 h), 92- and 135-kDa activities rose significantly above control levels. Doxycycline treatment significantly reduced the LPS-induced increase in 92-kDa but not 135-kDa gelatinolytic activities (Table 2).

**DISCUSSION**

We have previously demonstrated the upregulation of MMP-2 in vascular hyporeactivity to LPS as well as the protective effects of MMP inhibition in in vitro models of endotoxemia (21). However, the effect of in vivo administration of the MMP inhibitor, doxycycline, on vascular hyporeactivity caused by in vivo LPS was unknown. This study demonstrates that in vivo treatment with doxycycline protects against the LPS-induced development of vascular hyporeactivity to vasoconstrictors. In aortas from endotoxemic rats, we observed increased specific MMP-2 activity vs. control aortas (shown as an increase in MMP-2 activity per unit protein),

**Table 1.** $pEC_{50}$ and $E_{max}$ values of aortic contractile responses to phenylephrine

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<th>Control + Doxy</th>
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<td>$pEC_{50}$</td>
<td>7.44±0.06</td>
<td>7.21±0.07</td>
<td>6.73±0.17*</td>
<td>6.97±0.10</td>
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<td>$E_{max}$</td>
<td>3.27±0.11</td>
<td>3.20±0.04</td>
<td>1.46±0.24*</td>
<td>2.23±0.22*</td>
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Values are means ± SE; $n = 7$ samples/group. Doxy, doxycycline; LPS, lipopolysaccharide; $E_{max}$, maximum effect. *$P < 0.05$ vs. control and †$P < 0.05$ vs. LPS, one-way ANOVA, Newman-Keuls post hoc test.

Fig. 2. Top: representative immunoblot for inducible nitric oxide synthase (iNOS) in aortic homogenates. Bottom: quantification of iNOS level in aortic homogenates. *$P < 0.05$ vs. control by one-way ANOVA, Newman-Keul’s post hoc test; $n = 4$ aortas/group.

Fig. 3. A, top: representative zymogram for 72-kDa matrix metalloproteinase (MMP)-2 activity in aortic homogenates. A, bottom: quantification of 72-kDa MMP-2 activity in aortic homogenates. B, top: representative Western blot for 72-kDa MMP-2 protein in aortic homogenates. B, bottom: quantification of 72-kDa MMP-2 protein in aortic homogenates. C: ratio of 72-kDa MMP-2 activity to 72-kDa MMP-2 protein level. *$P < 0.05$ vs. control by one-way ANOVA, Newman-Keuls post hoc test. Bars are means ± SE; $n = 4$ homogenates/group.
In the plasma, we observed a significant upregulation of 92-kDa MMP-9 activity that was attenuated with doxycycline. This upregulation of both MMP-2 and -9 activities in the aorta and plasma, respectively, presents novel mechanisms that may contribute to vascular hyporeactivity during endotoxemia. The MMP inhibitor doxycycline was able to attenuate LPS-induced hyporeactivity to both PE and KCl, indicating a fundamental dysregulation of the smooth muscle contractile machinery that may in part be due to MMP activity. Doxycycline is a tetracycline class antibiotic that exhibits MMP inhibitory activity independent of its antibacterial effects (13). This is already seen at doses lower than the minimum effective concentration required for antibacterial action (13). We therefore used a subantimicrobial dose of doxycycline (4 mg/kg) to investigate its property as an MMP inhibitor.

Despite the large body of evidence implicating excess generation of NO as a central mediator in the development of vascular hypococontractility and hypotension in sepsis, few studies have examined the relationship between NO and MMPs. In a study of septic shock in rats using a cecal ligation and puncture model, MMP inhibition with a chemically modified tetracycline administered by gavage 1 and 24 h before the insult was able to reduce mortality and decrease plasma nitrate levels vs. controls measured at 0, 0.5, 1.5, and 24 h (26). In contrast, the NO scavenger diethyldithiocarbamate in vivo prolonged survival in LPS-treated mice and decreased MMP-2 and -9 protein level in the plasma (17). Some studies have also suggested that high concentrations of tetracyclines may decrease iNOS protein expression via destabilization of iNOS mRNA (1, 39), thus resulting in a decrease of excessive NO production. In the present study, iNOS protein was induced in the aorta from endotoxemic rats; however, doxycycline administration did not reduce iNOS expression. This indicates that the protective effect of MMP inhibition in our model of endotoxemia may be either downstream or independent of iNOS. Possible downstream fates of NO include its spontaneous reaction with superoxide to form peroxynitrite. In general, peroxynitrite biosynthesis, even independent of iNOS activation, can activate MMPs (30), including MMP-2 (47), by reacting with the cysteinyl sulfydryl group in the autoinhibitory propeptide domain, resulting in an active full-length 72-kDa enzyme. In rat vascular smooth muscle cells treated with LPS, interferon-γ, and phorbol 12-myristate 13-acetate, MMP-9 mRNA levels were increased via the NO/soluble guanylate cyclase pathway (27). However, the contribution of the NO/soluble guanylate cyclase-dependent pathway to LPS-induced vascular hyporeactivity and how this interacts with MMPs will require further studies.

In the plasma MMP-9 activity was increased and this was attenuated by doxycycline. The tertiary granules of polymorphonuclear leukocytes contain MMP-9 that is released upon LPS administration (28, 31). The precise role of MMP-9 in septic shock is, however, poorly understood. The upregulation of circulating MMP-9 activity may also contribute to vascular contractile dysfunction since MMP-9 knockout mice show similar to that previously described (21). In the plasma, we observed a significant upregulation of 92-kDa MMP-9 activity that was attenuated with doxycycline. This upregulation of both MMP-2 and -9 activities in the aorta and plasma, respectively, presents novel mechanisms that may contribute to vascular hyporeactivity during endotoxemia. The MMP inhibitor doxycycline was able to attenuate LPS-induced hyporeactivity to both PE and KCl, indicating a fundamental dysregulation of the smooth muscle contractile machinery that may in

### Table 2. MMP activities in plasma using gelatin zymography

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<th>Control</th>
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<tr>
<td>MMP-2</td>
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<tr>
<td>75 kDa</td>
<td>109±13</td>
<td>120±21</td>
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<td>72 kDa</td>
<td>1,205±277</td>
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<td>MMP-9</td>
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<td>135 kDa</td>
<td>99±39</td>
<td>1,495±278*</td>
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<td>92 kDa</td>
<td>195±82</td>
<td>1,750±560*</td>
<td>786±306†</td>
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Values are means ± SE; n = 4 samples/group. MMP, matrix metalloproteinase. *P < 0.05 vs. control (*) and vs. LPS †, one-way ANOVA, Newman-Keuls post hoc test.
resistance to endotoxic shock as evidenced by prolonged survival (9). In a clinical study increased plasma MMP-9 was found in septic patients and its level correlated with the severity of sepsis (36). Moreover, our laboratory has demonstrated a potential role for MMP-9 in cardiac contractile dysfunction in endotoxemia (23). Symptoms of vascular hyporeactivity in vivo may involve an interaction with both plasma MMP-9 and vascular MMP-2.

The apparent discordance between protein levels (as measured by immunoblot) and activity (as measured by zymography) of 72-kDa MMP-2 is an interesting finding. We have found similar results in our in vitro study (21). Although we did not analyze the aortic peroxynitrite content in the present study, this discordance may be attributable to peroxynitrite-induced activation of these enzymes. Peroxynitrite can cause posttranslational modifications to the MMPs, resulting in catalytically active enzyme without need for proteolysis-dependent activation (30, 47). Thus increased aortic peroxynitrite biosynthesis during endotoxemia (45) may have activated 72-kDa MMP-2 without requiring its conversion to the 64-kDa form by loss of the propeptide. Such activation could allow for MMP-2 specific activity to appear equal to or even higher when comparing LPS and control aorta (Fig. 3C), despite a loss in MMP-2 protein content in the former. Other explanations also exist for the observed discordance between zymography and immunoblot, such as possible epitope modification by peroxynitrite or protease activity, which may reduce the binding of primary antibody to MMP-2 and consequently the immunoblot results.

We could also observe similar results in our immunohistochemical analysis. In aortic cross sections from LPS-treated rats we observed a decrease in the immunohistochemical staining of MMP-2 vs. controls, thus suggesting a release of the active enzyme from vascular tissue (Fig. 4). Whether this event is a result of, or contributes to, vascular hyporeactivity remains to be examined. The possible MMP-2 release from cells (5, 21, 23, 40) may occur as a protective mechanism to diminish intracellular proteolytic activity or it may continue to have effects on vascular tone by proteolyzing targets outside of the cell. We recently investigated the role of the endothelium in enhanced vascular MMP-2 activity in in vitro models of endotoxin shock (4). We observed that the mechanism of MMP-induced vascular hyporeactivity stimulated by LPS in vitro is probably endothelium-dependent. Furthermore, some studies have demonstrated the direct vasodilatory effects of MMP-2 and -9 applied to isolated blood vessels in vitro, which may serve as another pathophysiological mechanism in endotoxic shock (6, 37).

In addition to the large body of evidence demonstrating extracellular effects of MMPs, work from our laboratory has demonstrated acute intracellular effects of MMPs as a result of enhanced oxidative stress (40). Specifically, it is shown that MMP-2 can degrade troponin I in cardiac myocytes, an intracellular regulator of cardiac contraction (48). Therefore, it is tempting to suggest that MMPs may act intracellularly to degrade susceptible proteins in vascular smooth muscle in septic shock. We are currently investigating the potential targets of MMPs in the vasculature during endotoxemia.

One important limitation of our study was the use of doxycycline as an MMP inhibitor. As it interacts with the catalytic zinc atom at the active site of several MMPs, it is, therefore, not selective for only MMP-2. However, it is known to preferentially inhibit MMP-2, -9, and -8 activities and is a much weaker inhibitor of MMP-1. Conversely, it does not inhibit MMP-3 or MMP-7 (13, 18, 29, 41). Future studies using more specific inhibitors of MMP-2 and/or -9 should be considered. Doxycycline might also exert some anti-inflammatory effects in this model of septic shock, thus decreasing plasma MMP-9 levels. We therefore cannot entirely exclude the possible contribution of MMP-9 in vascular hyporeactivity during endotoxemia. Gelatin zymography separates MMPs from any possible inhibitors; thus, zymography does not reflect the intracellular proteolytic balance between MMPs and any possible bound inhibitors of MMP activity.

These results reveal for the first time the effects of the most potent MMP inhibitor of the tetracycline class of antibiotics, doxycycline, on in vivo LPS-induced vascular hyporeactivity to vasoconstrictors. This information is important to understand the mechanisms behind the development of LPS-induced vascular hyporeactivity to vasoconstrictors in vivo. Targeting MMP-2 and MMP-9 may be a viable future option in the treatment of vascular hyporeactivity in endotoxic shock.

GRANTS

This study was funded by a grant from the Canadian Institutes of Health Research (CIHR) (FRN66953). J. Cena was supported by a studentship from the Alberta Heritage Foundation for Medical Research (AHFMR). A. K. Chow is supported by a Banting and Best Canada Graduate Scholarship (CIHR) and an AHFMR Incentive Award. M. Lalu was supported by a fellowship award from the CIHR. M. Bagdan was supported by an AHFMR summer studentship. M. Castro is supported by an AHFMR fellowship. R. Schulz is an AHFMR scientist.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES

MMP INHIBITION PROTECTS AGAINST VASCULAR HYPOREACTIVITY


