Peroxynitrite-induced myocardial injury is mediated through matrix metalloproteinase-2

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Abstract

Objectives: Peroxynitrite (ONOO\textsuperscript{-}) mediates in part both ischemia–reperfusion and pro-inflammatory cytokine-induced injury to the heart. As oxidants like ONOO\textsuperscript{-} are known to activate matrix metalloproteinases (MMPs), we examined whether they play a role in the detrimental action of ONOO\textsuperscript{-} in isolated perfused rat hearts. Methods: Hearts were isolated from Sprague–Dawley rats and perfused retrogradely with Krebs–Henseleit buffer under constant flow. Peroxynitrite (30 and 80 μM) was infused into the hearts for 15 min. The release of MMPs into the coronary effluent and level of MMPs in the myocardium were measured by gelatin zymography. Results: The main gelatinolytic activity in control effluent was 72-kDa corresponding to pro-MMP-2. Infusion of ONOO\textsuperscript{-} (80 μM) for 15 min caused a vasodilatation which peaked at 5 min and then converted into vasoconstriction by 15 min. It also caused a rapid increase in the release of 72-kDa activity within 10 min and a progressive decline in cardiac mechanical function. In contrast, decomposed ONOO\textsuperscript{-} caused no change in vascular tone, the release of 72-kDa activity or mechanical function. The MMPs inhibitor PD-166793 prevented the ONOO\textsuperscript{-}-induced loss in myocardial mechanical function. Detoxification of ONOO\textsuperscript{-} with glutathione prevented both the enhancement in coronary effluent 72-kDa activity and the decline in mechanical function. Conclusions: Acute cardiac toxicity induced by ONOO\textsuperscript{-} is mediated by MMP-2.

Keywords: Contractile function; Heart failure; Nitric oxide

1. Introduction

Nitric oxide (NO) and superoxide (O\textsubscript{2}\textsuperscript{-}) react at a diffusion-limit rate to form peroxynitrite (ONOO\textsuperscript{-}) [1]. As a potent oxidant species, ONOO\textsuperscript{-} is recognized to play a key role in many cardiac pathologies such as ischemia–reperfusion injury [2–5] and pro-inflammatory cytokine-induced myocardial dysfunction [6]. Indeed, antioxidants such as glutathione detoxify ONOO\textsuperscript{-} and protect hearts from ischemia–reperfusion injury [7]. Many biological molecules such as proteins [8], lipids [9], carbohydrates [10] and nucleic acids [11] can react with and are modified by ONOO\textsuperscript{-} and therefore are considered to be its targets. Many enzymes are inactivated and lose their function such as aconitase [12] and Na\textsuperscript{+}–K\textsuperscript{+}-ATPase [13] upon exposure to ONOO\textsuperscript{-}. In contrast, the latent forms of matrix metalloproteinases (MMPs) are known to be activated by oxidant species including ONOO\textsuperscript{-} [14,15]. MMPs are a family of zinc-containing endopeptidases with at least 20 members and are best recognized for their ability to degrade the extracellular matrix [16]. While all MMPs are synthesized as pro-enzymes (pro-MMPs), most of them are secreted from the cell as pro-MMPs and are activated pericellularly [17]. Breakage of the cystinyl sulphhydroly bond between a cysteine residue of the propeptide and the Zn\textsuperscript{2+} catalytic centre is necessary for the

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activation of the zymogen form [18]. This can be achieved by either proteolytic cleavage of part of the pro-peptide, resulting in an active enzyme with 8–10 kDa lower mass, by conformational changes induced by denaturing agents such as sodium dodecyl sulfate [18] or by oxidant stress molecules like ONOO⁻ [14]. For example, ONOO⁻ was shown to be a potent activator of pro-MMP-8 without causing a change in its molecular weight [14]. The activities of MMPs are also regulated by endogenous inhibitors such as the tissue inhibitors of metalloproteinases (TIMPs) [16].

Apart from roles of MMPs in long-term remodeling processes such as embryonic development, wound healing and cancer invasion [17], there is increasing evidence that some MMPs like MMP-2 can also rapidly regulate diverse cellular functions independent of their effects on the extracellular matrix [19–23]. This includes effects of MMP-2 on platelet aggregation [19], the control of vascular contractile tone through the proteolytic effects of MMP-2 on big endothelin-1 [20] and calcitonin gene related peptide [21] and attenuation of inflammatory signals by cleavage of monocye chemoattractant protein-3 [22]. We recently demonstrated that MMP-2 activity is acutely enhanced in isolated perfused rat hearts during reperfusion following ischemia and contributes in part to myocardial stunning [23]. The mechanism of this enhancement remains obscure. Because the time course of the acute enhancement in ONOO⁻ and MMPs in the heart in the first minute of reperfusion following ischemia [3,23] are similar, we hypothesized that ONOO⁻ activates MMPs in the myocardium and that the mechanical dysfunction of heart subjected to the infusion of exogenous ONOO⁻ is mediated in part by MMPs.

2. Methods

This investigation conforms to the Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care (revised 1993).

2.1. Materials

The supernatant from phorbol ester activated human fibroblast HT1080 cells (American Type Culture Collection, Rockville, MD), which contains large amounts of pro-MMP-2, MMP-2, pro-MMP-9 and MMP-9 was used as a standard. The MMP inhibitor PD-166793 was a kind gift from Parke-Davis (Ann Arbor, MI). All other agents were purchased from Sigma (St. Louis, MO). Peroxynitrite and decomposed ONOO⁻ were freshly prepared as previously described before each experiment [3]. Briefly, an ice-cold solution of NaNO₂ (2 M) and a mixed solution of nitric acid (11.1 M) and H₂O₂ (8.2 M) in two separate syringes were simultaneously discharged into a rapidly stirring solution of excess ice-cold NaOH (4.2 M). Decomposed ONOO⁻ was prepared by discharging the NaNO₂ solution and nitric acid–H₂O₂ mixture into an empty beaker on ice. After allowing 5 min to ensure complete decomposition of ONOO⁻, the same volume of NaOH solution as used for the preparation of ONOO⁻ was added. The solutions were filtered through Mn(IV)O₂ to remove excess H₂O₂. The concentration of ONOO⁻ and verification of decomposed ONOO⁻ was determined by UV spectroscopy (λmax=302 nm; ε=1670 M⁻¹cm⁻¹). Stock solutions were prepared by dilution in double distilled water and were kept in a light-shielded container during the infusion.

2.2. Heart preparation and perfusion

Male Sprague–Dawley rats (250–300 g) were used for the experiments. Hearts were rapidly excised from pentobarbital anesthetized rats and briefly rinsed by immersion into ice-cold Krebs–Henseleit solution. They were perfused via the aorta at a constant flow of 10 ml/min by means of a peristaltic pump (Buchler Instruments Inc, Fort Lee, NJ) with Krebs–Henseleit buffer at 37°C. The composition of the buffer was (in mM): NaCl (118), KCl (4.7), KH₂PO₄ (1.2), MgSO₄ (1.2), CaCl₂ (3.0), NaHCO₃ (25), glucose (11), EDTA (0.5) and it was continuously gassed with 95% O₂/5% CO₂ (pH 7.4).

Spontaneously beating hearts were used in all experiments. A water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle through an incision in the left atrium and through the mitral valve and the volume was adjusted to achieve an end diastolic pressure of 8–12 mmHg. A transducer was placed in the infusion line close to the heart to monitor coronary perfusion pressure. Coronary perfusion pressure, heart rate and left ventricular pressure were monitored on an IBM compatible computer using an MP100 system (BIOPAC Systems Inc, Santa Barbara, CA). Left ventricular developed pressure (LVDp) was calculated as the difference between systolic and diastolic pressures of the left ventricular pressure trace. The rate-pressure product (RPP) was calculated as the product of heart rate and left ventricular developed pressure. A water-jacketed glass chamber was positioned around the heart to maintain its temperature at 37°C. Stock solutions of various reagents were infused into the heart via a side-port proximal to the aortic cannula at a constant rate of 0.1 ml/min by a Gilson mini pump (Minipuls 3, Villiers Le Bel, France).

The intervals between thoracotomy and attachment of the heart to the perfusion system and between thoracotomy and beginning of stabilization period were less than 1 and 5 min, respectively. Using this perfusion protocol, hearts maintained a steady state of coronary flow, heart rate and left ventricular developed pressure for at least 80 min after stabilization.
2.3. Infusion of ONOO

Following 20 min of perfusion (stabilization period), the thromboxane mimetic U46619 (1–100 nM) was infused at a concentration sufficient to increase the coronary perfusion pressure to 100–150 mmHg from a baseline of 40 mmHg. The infusion of U46619 was maintained throughout the duration of the perfusion. After 15 min of U46619 infusion, when the increase in coronary perfusion pressure reached a plateau in all hearts, either decomposed ONOO⁻ or ONOO⁻ (30 and 80 µM each) were infused into the hearts for 15 min followed by a 15 min washout period. In pilot experiments, we did not see any effect of these concentrations of U46619 on MMP-2 release into the coronary effluent. Hearts were perfused for a total duration of 65 min and their mechanical function was recorded. Hearts were frozen immediately into liquid nitrogen temperature and stored at −80°C.

In separate groups of hearts, either PD-166793 (2 µM) or glutathione (GSH, 300 µM) were infused into the heart at the same time with U46619. S-nitroso-acetylpenicillamine (SNAP, 3 µM) was infused into a separate group of hearts for 15 min in a similar manner as ONOO⁻. Coronary effluent samples were collected at same points as in the ONOO⁻ group.

2.4. Protein concentration of the coronary effluent

The 6-ml coronary effluent samples were concentrated in Centricon-30 concentrating vessels (Amicon Inc, Beverly, MA). After concentration, the final volume of samples was estimated gravimetrically. Protein concentration in the concentrate was determined by bicinchoninic acid assay using bovine serum albumin as standard. The protein concentration in coronary effluent before concentration was calculated and expressed as micrograms per milliliter.

2.5. Preparation of heart extracts

Frozen hearts were crushed using a mortar and pestle at liquid nitrogen temperature and then homogenized by sonication in ice-cold 50 mM Tris–HCl (pH 7.4) containing 3.1 mM sucrose, 1 mM dithiothreitol, 10 µg/ml leupeptin, 10 µg/ml soybean trypsin inhibitor, 2 µg/ml aprotinin, and 0.1% Triton X-100. The homogenate was centrifuged at 10 000×g at 4°C for 10 min and the supernatant was collected and stored at −80°C.

2.6. Measurement of MMPs by gelatin zymography

Gelatin zymography was performed as previously described [23]. Briefly, concentrated coronary effluent samples were mixed with non-reducing sample loading buffer and applied to 8% polyacrylamide gel copolymerized with 2 mg/ml gelatin. Then, 1–2 µg of effluent concentrate or 40 µg of myocardial tissue extract were loaded in each lane. After electrophoresis, gels were rinsed in 2.5% Triton X-100 (3×20 min) to remove sodium dodecyl sulfate. Then the gels were washed twice in incubation buffer for 20 min each at room temperature. The composition of the incubation buffer was (in mM): Tris–HCl (50), CaCl₂ (5), NaCl (150) and 0.05% NaN₃. The gels were then kept in incubation buffer at 37°C overnight. After incubation, gels were stained in staining solution (2% Coomassie Brilliant blue G, 25% methanol, 10% acetic acid) for 2 h and then destained twice for 30 min each in destaining solution (2% methanol, 4% acetic acid). Zymograms were scanned using an HP 6100 scanner (Hewlett-Packard), and the band intensities were analyzed by Sigmagel software (version 1.0, Jandel Scientific). MMP activities were expressed as a specific activity per microgram of protein in either the coronary effluent or the myocardial extract.

2.7. Inhibition of MMPs by PD-166793 in vitro

To investigate the inhibitory profile of PD-166793 on the gelatinolytic activities of MMPs, PD-166793 (0.2–10 µM) was added to the incubation buffer during the overnight incubation period.

2.8. Statistical analysis

Data were expressed as mean±standard error of the mean (S.E.M.). One-way or two-way repeated measures ANOVA with Neuman Keuls as a post-hoc test or Student’s t-test was used for statistical analysis when appropriate. A value of P less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of ONOO⁻ on coronary vascular tone and cardiac mechanical function

Infusion of 30 µM ONOO⁻ caused vasodilatation evidenced as a decline in coronary perfusion pressure which peaked at 10 min, was maintained for the duration of the 15 min infusion and was reversed upon washout (Fig. 1A). Infusion of 80 µM ONOO⁻ caused a more rapid vasodilatation which peaked at 5 min, and then rapidly converted to vasoconstriction which was maintained even after washout of ONOO⁻. Only slight vasoconstriction, which was not statistically significant, was seen in decomposed ONOO⁻ treated hearts (Fig. 1A). The 15 min infusion of 80 µM, but not 30 µM ONOO⁻, also caused a slowly developing and significant depression of cardiac function.
mechanical function. This was due mainly to a decrease in left ventricular developed pressure (LVDP), accompanied by a slight but insignificant decrease in heart rate (Table 1). In contrast, hearts treated with decomposed ONOO\(^-\) showed no change in mechanical function (Fig. 1B). Fifteen minutes after stopping the infusion of 80 \(\mu\)M ONOO\(^-\), cardiac mechanical function remained significantly depressed compared with hearts treated with decomposed ONOO\(^-\) (Fig. 1B).

3.2. Effect of ONOO\(^-\) on coronary effluent and myocardial MMP-2 levels

In accordance with our previous study [23], all coronary effluents at baseline from aerobically perfused hearts showed a strong band of 72-kDa gelatinolytic activity (Fig. 2A), whereas only some effluents showed a much weaker band of 64-kDa activity (data not shown). While infusion of 30 \(\mu\)M ONOO\(^-\) did not increase 72-kDa gelatinolytic activity, infusion of 80 \(\mu\)M ONOO\(^-\) caused a rapid increase in this activity in the coronary effluent. This increase did not occur in the effluent from hearts infused with decomposed ONOO\(^-\) (Fig. 2A and B). The 72-kDa activity peaked at 10 min of ONOO\(^-\) infusion and subsequently declined. After washout, the release of gelatinolytic activity from the heart returned to baseline levels. Moreover, infusion of ONOO\(^-\) caused an increase in net protein release from the hearts (Fig. 2C), however, this became statistically significant only at 15 min infusion, after the peak increase in 72-kDa gelatinolytic activity (Fig. 2B). There was no difference over time in the protein concentration in the effluent from both decomposed ONOO\(^-\) and 30 \(\mu\)M ONOO\(^-\)-treated hearts (Fig. 2C).

There was no discernable change in tissue 72-kDa gelatinolytic activity levels between 80 \(\mu\)M ONOO\(^-\) or decomposed ONOO\(^-\)-treated hearts either at 10 min infusion or at the end of washout period (130±28 vs. 139±31 and 122±12 vs. 118±32 arbitrary units/\(\mu\)g protein, \(P>0.05\), \(n=6\), respectively).

### Table 1

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* \(P<0.05\) vs. baseline by one-way ANOVA with Neuman–Keuls post-hoc test.
Fig. 2. Time-dependent effects of ONOO⁻ (30 and 80 μM) on the release of gelatinolytic activity into the coronary effluent. (A) Representative zymogram of coronary effluent samples from ONOO⁻-treated hearts. Standard indicates HT1080 cell-conditioned medium. (B) Summary data quantifying 72-kDa activity from hearts infused either ONOO⁻ or decomposed (dec.) ONOO⁻. (C) Total protein concentration in the coronary effluent. Bar denotes infusion of ONOO⁻ or dec. ONOO⁻. For both panels (B) and (C), the differences between 80 μM ONOO⁻ and decomposed ONOO⁻ are P<0.05 by two-way repeated measures ANOVA, n=6 in each group.
3.3. Effect of glutathione on MMP-2 release and myocardial function of ONOO⁻-treated hearts

The peroxynitrite scavenger glutathione (300 μM) blocked the increase of 72-kDa activity in the coronary effluent (Fig. 3A), and prevented the decline in cardiac mechanical function caused by infusion of 80 μM ONOO⁻ (Fig. 3B). In accordance with our previous results [7], ONOO⁻ caused only prolonged vasodilatation in the presence of glutathione (data not shown).

3.4. Effect of MMP inhibitor on MMP-2 release and myocardial function of ONOO⁻-treated hearts

PD-166793, when added to the zymography incubation buffer, concentration-dependently inhibited the 72-kDa activity of a 35 min perfusion coronary effluent sample from an aerobically perfused heart (Fig. 4A). PD-166793 did not alter the coronary vascular response to ONOO⁻, neither did it have an effect on the concentration of ONOO⁻ during its in vitro incubation with ONOO⁻ for 15 min at room temperature (data not shown). The hearts co-infused with both ONOO and PD-166793 still showed increased release of 72-kDa gelatinolytic activity (Fig. 4B). This is expected because of the ready dissociation of the complex between MMP-2 and PD-166793 during electrophoretic conditions (data not shown). PD-166793 (2 μM) abolished the depression of cardiac mechanical function induced by 80 μM ONOO⁻ (Fig. 4C).

3.5. Effect of SNAP on coronary vascular tone and MMP-2 release

ONOO⁻ reacts with endogenous thiols such as glutathione forming nitrosothiol intermediates which then release nitric oxide [24,25]. To verify whether the enhancement of 72-kDa activity in the coronary effluent by ONOO⁻ was due to vasodilatation or due to NO formation, we used the nitrosothiol NO donor S-nitroso-acetylpenicillamine (3 μM, n=5) to induce vasodilatation to a similar level as seen in ONOO⁻-treated hearts and did not find any change in MMP activity in the coronary effluent. Infusion of SNAP caused a rapid and reversible vasodilatation without having any effect on mechanical function (Fig. 5A). However, it caused no change in the release of 72-kDa gelatinolytic activity (Fig. 5B).

4. Discussion

We have shown here that infusion of authentic ONOO⁻ caused a rapid and significant increase in 72-kDa gelatinolytic activity into the coronary effluent which preceded the decline in myocardial contractile function. Detoxification of ONOO⁻ with glutathione prevented not only the increase in 72-kDa activity but also the myocardial dysfunction. Inhibition of MMP-2 with PD-166793 protected hearts from ONOO⁻-induced myocardial depression. Our findings suggest a novel role of MMP-2 in mediating ONOO⁻-induced injury to the heart.

In spite of its short half-life, the heart was exposed to a constant concentration of ONOO⁻ by its continuous infusion into the perfusion buffer via a side-port immediately proximal to the aortic cannula. In a similar model as used here, Vila et al. [26] gave bolus injections of 100 μM ONOO⁻ over 30 s into isolated rat hearts and found that it caused vasodilatation. This vasodilatory response to ONOO⁻ showed rapid desensitization upon repeat injection. Similarly, we found that continuously infusion of 80 μM ONOO⁻ caused an initial vasodilatation which then converted into vasoconstriction. This effect of ONOO⁻ was also concentration-dependent as 30 μM ONOO⁻ infusion showed only prolonged vasodilatation (Fig. 1A). No discernible change of coronary perfusion pressure was observed in hearts treated with decomposed ONOO⁻. It is plausible that a nitrosothiol NO donor is
Fig. 4. (A) Inhibitory profile of PD-1666793 on MMP activities in a coronary effluent sample from aerobically perfused rat hearts taken at 35 min perfusion. (B) Effect of 2 μM PD-1666793 on MMP-2 release stimulated by 80 μM ONOO⁻ infusion into the coronary effluent. (C) Effect of 2 μM PD-1666793 on mechanical function measured as rate-pressure product from hearts subjected to 80 μM ONOO⁻. * P<0.05 vs. ONOO⁻ by two-way repeated measures ANOVA, n=6 in each group.
effect of ONOO\(^{-}\) due to coronary vasoconstriction, because we have found that there was no relation between coronary vasoconstriction and MMP-2 release (Wang and Schulz, unpublished results). Thus the pro-oxidant property of ONOO\(^{-}\) appears to be responsible for the enhancement in 72-kDa activity.

That we did not observe any concomitant loss in tissue 72-kDa activity following ONOO\(^{-}\) infusion may have several reasons: (a) our previous observation of this in ischemia–reperfusion injury [23] is because that is a stronger insult to the heart which indeed resulted in a greater release of 72-kDa activity; (b) ischemia–reperfusion results in oxidative stress due to endogenous ONOO\(^{-}\) formed directly within cardiomyocytes; and (c) the release of 72-kDa activity appears to be a subtle yet early response to an intravascular oxidative stress.

ONOO\(^{-}\) is able to enhance MMPs activity by several means. As it is recognized for its ability to oxidize thiols [29], ONOO\(^{-}\) might cause oxidative modification of the cysteine residue in the pro-peptide domain. Perturbation of the cysteine–zinc bond, the so-called cysteine switch, is suggested to underlie the activation mechanism of pro-MMPs [18]. The zymogen form of purified human neutrophil collagenase (pro-MMP-8) was activated by authentic ONOO\(^{-}\) in a concentration range similar to what was used here [14] without causing a change in its molecular weight. It was also speculated that nitration of tyrosine residues in pressure (left panel) and mechanical function (right panel). (B) Effect of SNAP on MMP-2 release into coronary effluent. n=5 each.

produced by the reaction of endogenous tissue thiols with ONOO\(^{-}\) which then initiates the vasorelaxation [26].

In accordance with our previous report [23], the main gelatinolytic activity in coronary effluent from the aerobically perfused heart is 72-kDa, corresponding to pro-MMP-2. We did not see any 92-kDa gelatinolytic activity (MMP-9) either in coronary effluent or heart homogenate even with longer (72 h) incubation of the zymograms. This reflects the notion that MMP-9 is primarily found in neutrophils [27] and macrophages [28]. A rapid and significant increase in 72-kDa activity was observed in coronary effluent from hearts exposed to 80 \(\mu\)M ONOO\(^{-}\). Moreover, this enhancement was an early event induced by ONOO\(^{-}\) and unrelated to a general increase in the level of myocardial proteins in the coronary effluent, which peaked several minutes later (Fig. 2). Neither was it related to the formation of a nitrosothiol intermediate as the release of MMP-2 activity was not stimulated by \(S\)-nitrosocetylpenicillamine, a nitrosothiol which nonetheless caused a similar decrease in coronary perfusion pressure as ONOO\(^{-}\). Furthermore, exogenously supplied glutathione prevented the increase in coronary gelatinolytic activity and protected the heart from ONOO\(^{-}\)-induced myocardial dysfunction at the same concentration at which it protected hearts from endogenous ONOO\(^{-}\)-mediated injury caused by ischemia and reperfusion [7]. Nor is this

Not surprisingly, Owens et al. [31] observed an inhibition of MMP-2 activity with high concentrations of ONOO\(^{-}\) (\(\geq\)250 \(\mu\)M). It is compatible with the notion, as shown here, that a low level of oxidant stress may activate certain enzymes such as MMPs, whereas higher levels of oxidant stress by ONOO\(^{-}\) could incur further protein modifications (i.e. thiol oxidation, nitrosylation, nitrotyrosine formation) which result in the inhibition of enzyme activity [12,32]. Very high concentrations of ONOO\(^{-}\) (\(\geq\)500 \(\mu\)M) are able to directly induce protein fragmentation in cell-free systems [30,31,33].

The mechanism of myocardial injury induced by MMPs remains unclear. Apart from the extracellular matrix, little is known about other targets of MMPs [16]. Recently, myosin heavy chain was found to be susceptible to degradation by MMP-2 in vitro and evidence for degradation fragments were found in hearts from patients with dilated cardiomyopathy [34]. We have recent evidence

Fig. 5. (A) Effects of 3 \(\mu\)M SNAP or vehicle on coronary perfusion pressure (left panel) and mechanical function (right panel). (B) Effect of SNAP on MMP-2 release into coronary effluent. n=5 each.
showing that a contractile protein regulatory element, troponin I, is susceptible to proteolytic cleavage by MMP-2 and that MMP-2 is co-localized with troponin I in ischemic–reperfused hearts [35]. Moreover, inhibitors of MMPs prevented the degradation of troponin I in ischemic–reperfused hearts as well as improved the recovery of mechanical function [35]. Whether contractile protein elements such as myosin or troponin I are degraded during ONOO−-induced myocardial injury requires further study.

Peroxynitrite is implicated in the pathogenesis of several cardiovascular pathologies including ischemia–reperfusion injury [3–5], pro-inflammatory cytokine-induced myocardial contractile failure [6], doxorubicin-induced cardiac toxicity [36], autoimmune-induced myocarditis [37], acute allograft rejection [38] and cardiomyocyte apoptosis [39] and atherosclerosis [40]. Interestingly, MMPs have also been implicated in many cardiovascular diseases, particularly, ischemia–reperfusion injury (both acute [23], and chronic [41]), heart failure [42] and the vascular derangement in atherosclerosis [43]. We speculate that enhancement of ONOO− generation precedes the activation of MMPs, and therefore some of the detrimental effects of ONOO− are mediated by activation of these proteases. Indeed, antioxidant therapy using N-acetyl-cysteine prevented gelatinase activation in atherosclerotic aorta of hypercholesterolemic rabbits [44]. Specific drugs targeting of peroxynitrite and/or MMPs could prove useful therapeutic regimens in the treatment of these diseases.

In summary, we have demonstrated that ONOO− rapidly increased the release of MMP-2 activity in the coronary effluent. Inhibition of MMP activity or detoxifying ONOO− with glutathione protected the heart from ONOO−-induced myocardial injury. This provides a new insight into the pathological mechanism of myocardial dysfunction where ONOO− biosynthesis is enhanced.

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