Mechanical traumatic injury without circulatory shock causes cardiomyocyte apoptosis: role of reactive nitrogen and reactive oxygen species

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Tao, Ling, Hui-Rong Liu, Feng Gao, Yan Qu, Theodore A. Christopher, Bernard L. Lopez, and Xin L. Ma. Mechanical traumatic injury without circulatory shock causes cardiomyocyte apoptosis: role of reactive nitrogen and reactive oxygen species. Am J Physiol Heart Circ Physiol 288: H2811–H2818, 2005. First published February 4, 2005; doi:10.1152/ajpheart.01252.2004.—Apoptotic cell death plays a critical role in tissue injury and organ dysfunction under a variety of pathological conditions. The present study was designed to determine whether apoptosis may contribute to posttraumatic cardiac dysfunction, and if so, to investigate the mechanisms involved. Male adult mice were subjected to nonlethal traumatic injury, and cardiomyocyte apoptosis, cardiac function, and cardiac production of reactive oxygen/nitrogen species were determined. Modified Noble-Collip drum trauma did not result in circulatory shock, and the 24-h survival rate was 100%. No direct mechanical traumatic injury was observed in the heart immediately after trauma. However, cardiomyocyte apoptosis gradually increased and reached a maximal level 12 h after trauma. Significantly, cardiac dysfunction was observed 24 h after trauma in the isolated perfused heart. This was completely reversed when apoptosis was blocked by administration of a nonselective caspase inhibitor immediately after trauma. In the traumatized hearts, reactive nitrogen species (e.g., nitric oxide) and reactive oxygen species (e.g., superoxide) were both significantly increased, and maximal nitric oxide production preceded maximal apoptosis. Moreover, a highly cytotoxic reactive species, peroxynitrite, was markedly increased in the traumatic heart, and there was a significant positive correlation between cardiac nitrotyrosine content and caspase 3 activity. Our present study demonstrated for the first time that nonlethal traumatic injury caused delayed cell death and that apoptotic cardiomyocyte death contributes to posttrauma organ dysfunction. Antiapoptotic treatments, such as blockade of reactive nitrogen oxygen species generation, may be novel strategies in reducing posttrauma multiple organ failure.

apoptosis; nitric oxide; superoxide

MECHANICAL TRAUMA, such as that induced by motor vehicle crashes, represents a major medical and economic problem in the United States. As a result of advanced prehospital care and regional trauma systems development, fewer critically injured patients are dying at the scene of the accident, and more are surviving the first 24 h of their trauma center care. Therefore, identifying the mechanisms responsible for posttraumatic organ dysfunction and searching for therapeutic strategies to prevent secondary organ injury after trauma is critical in reducing overall trauma morbidity and mortality. Several recently published clinical reports (26, 30, 35, 37) have suggested that blunt chest trauma induces myocardial infarction and secondary cardiac dysfunction even in the absence of coronary artery dissection. However, the mechanisms responsible for this trauma-induced cardiac injury have not been identified.

Apoptosis is a special form of cell death that differs from necrosis in many aspects. One of the most widely recognized biochemical features of apoptosis is the activation of a class of cysteine proteases known as caspases (7, 33). Cells possess multiple caspases, which may work in a cascade fashion. The redundancy may serve to amplify and accelerate the response as well as to provide multiple mechanisms for success (29, 39). Two pathways have been identified that activate caspases: one activated through a cell surface signal leading to caspase 8 (and/or 10 in some cell types) activation and another more complicated pathway involving the mitochondria and resulting in caspase 9 activation. Activation of these initiating caspases then activates downstream executive caspases (e.g., caspase 3) and results in apoptotic cell death. Although substantial evidence now exists that apoptotic cell death plays a critical role in tissue injury and organ failure under a variety of pathological conditions, whether or not apoptosis may contribute to the secondary organ injury after mechanical trauma remains largely unknown.

Therefore, the aims of the present study were 1) to investigate whether a nonlethal mechanical trauma may result in cardiomyocyte apoptosis; and, if so, 2) to determine whether myocardial apoptosis may contribute to posttrauma cardiac dysfunction; and 3) to delineate potential mechanisms that are responsible for posttrauma myocardial apoptosis and cardiac dysfunction.

MATERIALS AND METHODS

Mice traumatic injury. Experiments were performed in adherence with the National Institutes of Health Guidelines on the Use of Laboratory Animals and were approved by the Thomas Jefferson University Committee on Animal Care. Male adult C57B16/J mice were anesthetized with pentobarbital sodium (40 mg/kg) and their tracheas were cannulated with polyethylene (PE)-50 tubing to ensure a patent airway. The mice were then placed in a Noble-Collip drum, a 12-in. diameter plastic wheel with internal shelves on which a rat is traumatized as the wheel is rotated. Our pilot experiments demonstrated that a nonlethal, fully recoverable traumatic injury was achieved when anesthetized mice were subjected to a total of 200 revolutions at a rate of 40 rpm. This procedure was used in all experiments presented in this study. After completion of the traumatic procedure, mice were placed in a warmed chamber and killed at the time points specified in RESULTS. In some animals, a PE-10 catheter...
filled with heparinized 0.9% NaCl solution was inserted into the left common carotid artery immediately after trauma for recording arterial blood pressure with a PowerLab data acquisition system. Sham trauma rats were subjected to the same revolution but the animals were taped on the inner wall of drum, thus avoiding traumatic injury. **Determination of myocardial apoptosis with DNA ladder formation.** Myocardial apoptosis was qualitatively analyzed by detection of DNA fragmentation (DNA ladders, n = 4–6/group), a hallmark of apoptosis as described previously (10, 17, 32). In brief, at the end of the experiment, the free wall of the left ventricle was isolated, minced while thawing, and homogenized. Tissue was digested with proteinase K at 56°C overnight and then incubated with DNA-free RNase at 37°C for an additional hour. Digested tissues were precipitated and centrifuged, and supernatants containing DNA were precipitated and centrifuged again. The resulting DNA pellets were washed and dissolved in DNA hydration solution. DNA electrophoresis was carried out at 60 V for 1-2 h. DNA ladder formation was visualized with a Kodak Image Station 400.

**TUNEL Assay.** To determine myocardial apoptosis in a quantitative manner, the hearts were perfused first with 0.9% NaCl for 5 min and then with 4% paraformaldehyde in PBS (pH 7.4) for 20 min. Four longitudinal sections from the free wall of the left ventricle were cut and further fixed in 4% paraformaldehyde in PBS for 24 h at room temperature. Fixed tissues were embedded in a paraffin block and two slides at 4- to 5-μm thickness were cut from each tissue block. Immunohistochemical procedures for detecting apoptotic cardiomyocytes were performed by using an apoptosis detection kit (Boehringer-Mannheim, Ridgefield, CT) according to the manufacturer’s instructions. An additional staining was performed with monoclonal anti-sarcomeric actin. This staining enables the identification of myocytes and, therefore, a distinction between myocyte nuclei and nuclei of other cells in cardiac tissue. After being rinsed with PBS, slides were coversoned with mounting medium containing DAPI to permit total nuclei counting.

With the use of a ×20 objective, the tissue slide was digitally photographed with a QICAM-Fast Digital Camera mounted onto an Olympus BX51 fluorescence microscope. Total nuclei (DAPI staining, blue) and the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) positive nuclei (green) in each field were counted by IP Lab Image Analysis Software (Version 3.5; Scanalytics, Fairfax, VA) with a custom-made script (by Ken Anderson, Bio Vision Technologies, North Exton, PA). The index of apoptosis (number of TUNEL-positive nuclei/total number of nuclei ×100) was automatically calculated and exported to Microsoft Excel for further analysis. Results from different fields taken from the same animal were averaged and counted as one sample.

**Determination of myocardial apoptosis by caspase 3 activation.** Cardiac caspase 3 activity was performed by using caspase colorimetric assay kits (Chemicon International, Temecula, CA) as described in our previous study (17). In brief, myocardial tissue was homogenized in ice-cold lysis buffer for 30 s using a PRO 200 homogenizer. The homogenates were centrifuged for 5 min at 10,000 g at 4°C, supernatants were collected, and protein concentrations were measured by the bicinchoninic acid method (Pierce Chemical, Rockford, IL). To each well of a 96-well plate, supernatant containing 200 μg of protein was loaded and incubated with 25 μg Ac-DEVD-NAp, caspase 3 specific substrate, for 1.5 h. Na2EDTA and the free pNA was quantitated by using a SpectraMax-MaxPlus microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) at 405 nm.

**Determination of cardiac function in the isolated perfused heart.** At 24 h after trauma, animals were reanesthetized (40 mg/kg ip) and heparinized with sodium heparin (1,000 U/kg ip). Ten minutes after heparin injection, a midsternal thoracotomy was performed, and the heart was rapidly excised and placed into ice-cold Krebs-Henseleit buffer solution consisting of (in mM): 118 NaCl, 4.75 KCl, 1.19 KH2PO4, 1.19 MgSO4·7H2O, 2.54 CaCl2·2H2O, 25 NaHCO3, 0.5 EDTA, and 11 glucose. Within 30 s, the heart was mounted onto a nonrecirculation Langendorff heart perfusion apparatus (Radnoti Glass Technology, Monrovia, CA). The heart was perfused in a retrograde fashion via the aorta at a constant pressure of 70 mmHg with Krebs-Henseleit solution oxygenated with 95% O2-5% CO2 to maintain pH 7.4 at 37°C. Hearts were paced at 420 beats/min with a Grass Stimulator and coronary flow (CF) was measured via an in-line flow probe connected to an ultrasonic flow meter (Transonic Systems, Ithaca, NY).

To assess contractile function, a latex balloon was inserted into the left ventricular cavity through the mitral orifice and connected to a pressure transducer (model CDXIII; Cobe, Lakewood, CO). The balloon was initially inflated with saline to produce an end-diastolic pressure of 8 to 10 mmHg, which is on the plateau of the Starling curve for this preparation. Left ventricular pressure (LVP) and CF were concurrently recorded in a Dell computer via a data acquisition system (PowerLab; ADInstruments, Milford, MA). The left ventricular (LV) systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), LV developed pressure (LVDVP = LVSP − LVEDP), the maximal positive and negative values of the instantaneous first derivative of LVP (+dP/dt max and −dP/dt max), heart rate, and CF were obtained by using computer algorithms and an interactive videographics program (Chart V5.0 for Windows, PowerLab).

**Determination of NO + NO2 + NO3 content in cardiac tissue.** The free wall of the left ventricle was isolated and rinsed, homogenized in deionized water (1:10, wt/vol), and centrifuged at 14,000 g for 10 min. The tissue nitric oxide (NO) and its in vivo metabolic products (NO2 and NO3) in the supernatant, collectively known as NOx, were determined by using a chemiluminescence NO detector (Siever 280i NO Analyzer) as described in our previous study (10).

**Determination of myocardial superoxide generation.** Myocardial superoxide content was determined by lucigenin-enhanced luminescence as described previously (8, 14, 40) and modified in our recent study (31). In situ superoxide detection was performed with dihydroethidium (DHE; Molecular Probes) staining as described previously (23, 44).

**Quantitation of tissue nitrotyrosine content.** Cardiac nitrotyrosine, a footprint of in vivo reactive nitrogen species (e.g., ONOO−) formation, was determined by using an ELISA procedure as reported in our recent study (11). In brief, the free wall of left ventricle was separated and homogenized in ice-cold PBS. The homogenates were centrifuged, the supernatants collected, and protein concentrations were determined by the BCA method. A nitrated protein solution was prepared and diluted for use as a standard. These standard samples, along with tissue samples from hearts, were applied to disposable sterile ELISA plates and incubated overnight with primary antibody. The secondary antibody was added, and the peroxidase reaction product was generated by using O-phenylenediamine dihydrochloride (OPD) solution. The optical density was measured at 430 nm with a SpectraMax-MaxPlus microplate spectrophotometer. The nitrotyrosine content in tissue samples was calculated by using standard curves generated from nitrated BSA containing known amounts of nitrotyrosine and expressed as nanomoles per gram protein.

**Statistical analysis.** Time and group differences were determined by two-way ANOVA for repeated measures. For nonrepeated measures, data were subjected to ANOVA followed by the Scheffe’s correction for post hoc t-test comparison. Probabilities of 0.05 or less were considered to be statistically significant.

**RESULTS**

**Mean arterial blood pressure, gross organ injury, and survival rate.** The mild traumatic procedure (i.e., 200 revolutions at a rate of 40 rpm) used in this experiment did not induce typical circulatory shock. Mean arterial blood pressure (MABP) was transiently reduced immediately after trauma and quickly returned to a normal level (Fig. 1). There was mild...
splanchnic vascular engorgement but no signs of bowel ischemia or serosanguinous ascites were observed. Gross liver and kidney injury were observed in 3 and 4 of the 17 mice studied, respectively. However, no signs of direct mechanical damage, such as pericardial bleeding or contusion, was observed in the hearts. The mice were observed to be normally active in their cages, drinking almost immediately after regaining mobility and eating within 2–3 h. The 24-h survival rate was 100%.

**Traumatic injury caused significant myocardial apoptosis.** Our initial observations demonstrated that the mild mechanical trauma did not result in primary injury in the heart. To investigate whether mechanical trauma may result in secondary myocardial injury, as has been reported for burn trauma (16), the heart was removed at different time points after trauma, and cardiomyocyte apoptosis was determined. As illustrated in Fig. 2, TUNEL-positive cells were not increased when mice were killed immediately after the completion of trauma (time 0), but gradually increased thereafter. Apoptotic cell death reached a maximal level at 12 h after trauma and remained at a significantly high level 24 h after trauma.

DNA ladder formation is a hallmark of apoptotic cell death. To obtain more evidence that traumatic injury causes cardiomyocyte apoptosis, mice were subjected to trauma as described above and hearts were removed 12 h after trauma. As illustrated in Fig. 3A, myocardial tissue obtained from mice subjected to sham trauma showed no DNA fragmentation (lanes 2 and 4, S). In contrast, clear DNA ladder formation was observed in myocardial tissue from mice subjected to nonlethal traumatic injury (lanes 3 and 5, T). To ensure that DNA ladder formation in traumatic hearts is attributed primarily to residential cells rather than foreign cells, myocardial tissue was processed with immunofluorescence, and cardiomyocytes were identified with a monoclonal antibody against α-actin. As illustrated in Fig. 3B, there were no TUNEL-positive nuclei detected in samples isolated from mice subjected to sham trauma. However, significant cardiomyocytes were labeled TUNEL positive in samples from mice subjected to traumatic injury without circulatory shock. To obtain more evidence in a specific and quantitative manner, myocardial caspase-3, a final common pathway in caspase-dependent apoptosis, was determined. As summarized in Fig. 4, caspase-3 was markedly activated as evidenced by strong immunostaining with an antibody against activated caspase-3 (Fig. 4A) and a 2.3-fold increase in caspase-3 activity determined by a colorimetric kit (Fig. 4B). These results demonstrated for the first time that traumatic animals without circulatory shock suffered from significant cell loss in an organ that cannot reproduce functional cells.

To investigate whether mild mechanical trauma may also induce heart injury by necrotic cardiomyocyte death, serum cardiac Troponin-I (cTrI), a cardiac specific protein released when the cardiomyocyte membrane is disrupted, was determined by using a high-sensitivity cTrI ELISA kit by following the manufacturer’s instructions (Life Diagnostics, West Chester, PA). As illustrated in Fig. 5, the changes in serum cTrI level followed a time course opposite to the apoptosis described above. Specifically, serum cTrI was slightly increased immediately after trauma (P < 0.05) and quickly returned to a level that was not significantly different from the value obtained in sham trauma mice. To validate our method and to obtain a positive control, three animals were subjected to 30 min of myocardial ischemia and 3 h of reperfusion, and serum cTrI was determined. Consistent with the results published by other investigators, myocardial ischemia/reperfusion resulted in a remarkable increase in serum cTrI level, indicating that a significant necrotic cell death occurred under this pathological condition. Taken together, these results clearly demonstrated that mechanical trauma used in the present experiment only resulted in a transient and rather mild necrotic cardiomyocyte injury that occurs immediately after trauma as a result of direct mechanical force applied to the heart during the trauma procedure. However, a secondary heart injury dominated by apoptotic cell death occurred 3 to 6 h after trauma.

**Traumatic injury resulted in significant cardiac dysfunction 24 h after trauma.** The mild mechanical trauma failed to induce a typical traumatic shock and the MABPs measured 3 to 24 h posttrauma and thereafter were normal. Because the MABP is regulated by multiple neuronal and humoral factors in vivo, a normal MABP does not necessarily mean that cardiac contractile function is normal. To determine whether trauma-induced cardiomyocyte apoptosis may result in cardiac dysfunction when other compensatory factors are eliminated, hearts were isolated 24 h after traumatic injury and perfused in vitro using a Langendorff apparatus. As illustrated in Fig. 6A and summarized in Fig. 6B and Fig. 7, hearts isolated from traumatic mice

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**Fig. 1.** Time course of mean arterial blood pressure (MABP) changes after modified Noble-Collip trauma in anesthetized mice. N = 7–9 mice/group. **P < 0.01 vs. sham control.

**Fig. 2.** Time course of myocardial apoptosis determined by TUNEL after nonlethal mechanical trauma. n = 5–7 Mice/group; **P < 0.01 vs. sham control.
exhibited a lower LVDP, reduced $+\frac{dP}{dt_{\text{max}}}$, and decreased $-\frac{dP}{dt_{\text{max}}}$ compared with hearts from sham trauma mice. Most interestingly, treatment with Z-VAD-FMK (a broad-spectrum caspase inhibitor, 4 mg/kg iv) (41) immediately after trauma, completely blocked trauma-induced cardiac dysfunction, although administration of this compound had no homodynamic effect in sham animals (data not shown). These results demonstrated that traumatic-cardiomyocyte apoptosis is a critical contributor to posttrauma cardiac dysfunction.

Mechanisms responsible for posttrauma cardiomyocyte apoptosis. Having demonstrated that mechanical trauma causes significant cardiomyocyte apoptotic cell death and that apoptosis contributes to posttrauma cardiac dysfunction, we further determined the mechanisms by which mechanical trauma may result in cardiomyocyte apoptosis. A recent study by White et al. (38) demonstrated that expression of inducible NO synthase (iNOS) and overproduction of NO are responsible for burn trauma-induced cardiomyocyte apoptosis. To investigate whether reactive nitrogen and/or oxygen species may contribute to mechanical trauma-induced cardiomyocyte apoptosis, three additional studies were performed in which iNOS expression, NO and superoxide ($O_2^-$) production, and peroxynitrite (ONOO$^-$) formation were determined. As illustrated in Fig. 8, clear iNOS expression was detected in cardiac tissue 3 h after traumatic injury. Consistently, cardiac NO content gradually increased after trauma and reached a maximal level 6 h after trauma. In a recent study, we demonstrated that NO, even at an extremely high concentration (3 orders of the magnitude of its maximal vasodilatory concentration), failed to induce significant apoptosis in cultured cardiomyocytes when cell permeable SOD mimics were added together with NO (Ma et al., unpublished observation). This result strongly suggests that it is the secondary reaction product of NO and $O_2^-$, not NO itself, that is responsible for high concentration NO induced cellular injury. To determine whether mechanical trauma may increase cardiac $O_2^-$ production thus facilitating the generation of toxic reactive nitrogen species, hearts were isolated 6 h (when maximal NO production occurs) after trauma and $O_2^-$ production was detected by the lucigenin-enhanced luminescence method. As summarized in Fig. 9B, cardiac $O_2^-$ production was markedly increased in traumatic hearts when compared with sham-operated hearts. To further determine the cellular origin of $O_2^-$ production in traumatic hearts (i.e., cardiomyocytes vs. inflammatory cells), cardiomyocyte $O_2^-$ production was determined by DHE staining. DHE reacts with superoxide anions to form ethidium bromide, which in turn intercalates with DNA to provide nuclear fluorescence as a marker of superoxide anion generation. As shown in Fig. 9A, the intensity of DHE staining in cardiac samples obtained from traumatic mice was markedly higher compared with staining intensity in the nontrauma myocardium.

Having demonstrated that both NO and $O_2^-$ production was significantly increased in traumatic cardiomyocytes, we further...
determined whether mechanical traumatic injury may increase cardiac formation of peroxynitrite, a highly toxic reactive nitrogen species that is generated by reaction of NO and O$_2$ at the diffusion-limited rate (3 times faster than its detoxification reaction with SOD) (2). As summarized in Fig. 10A, the tissue nitrotyrosine level (a footprint of in vivo peroxynitrite formation) determined 6 h after trauma, was dramatically increased. Moreover, a strong positive correlation between nitrotyrosine content and caspase-3 activity was observed (Fig. 10B).

**DISCUSSION**

We have made several novel observations in the present study. First, we have demonstrated that mechanical trauma resulted in significant cardiomyocyte apoptosis 6 h after trauma when animals recovered to a grossly “normal” condition. This is a completely different form of heart injury than that caused by direct force applied to the heart (e.g., coronary arterial dissertation and cardiac contusion) which occurs immediately...
after trauma (primary heart injury). Second, we have provided direct evidence that cardiomyocyte apoptosis contributes to posttraumatic cardiac dysfunction and that anti-apoptotic therapy might be an effective strategy to prevent or attenuate postinjury secondary organ failure. Third, we have demonstrated that the production of reactive nitrogen species and reactive oxygen species were both markedly increased in the traumatic heart. Maximal NO production preceded maximal apoptotic cell death, and caspase 3 activity was positively correlated with nitrotyrosine content in the traumatic heart. These results strongly suggest that protein nitrination is likely responsible for this trauma-induced cardiac apoptosis.

Postinjury multiorgan failure is a major medical challenge and causes a substantial economical burden in our society. Whereas the Noble-Collip drum-induced traumatic shock model has been used by numerous investigators to mimic lethal traumatic injury caused by accidents, such as motor vehicle crash, whether a modest nonlethal trauma which occurs more commonly in car accidents and falls may cause a secondary cell death and tissue injury has not been previously studied. Although it is expected that a severe trauma may induce direct mechanical heart injury (primary injury), what is truly intriguing is that mild trauma that does not cause direct mechanical heart injury and circulatory shock resulted in significant cardiomyocyte apoptotic cell death a few hours after trauma, a type of injury that has never been previously reported. Moreover, although this type of cell death (i.e., apoptosis) likely occurs in multiple organs, we believe that cardiac (and brain cell) apoptosis deserves the highest attention because these tissues are terminally differentiated and any amount of cell loss will have adverse functional consequences.
Apoptosis may contribute to cardiac dysfunction by several possible mechanisms. Loss of cardiomyocytes leads to loss of cardiac mass and hence diminished pump power. Loss of cardiomyocytes may also result in electrical conduction inhomogeneity that may lead to arrhythmias. Finally, apoptosis may lead to cardiac remodeling due to realignment of neighboring cardiomyocytes. This latter mechanism is unique to the heart, in which function is extremely dependent on optimal geometrical and structural alignment. Thus apoptosis, even if limited in scope, may result in widespread mechanical and electrical disturbances. These possibilities are further supported by studies in which inhibition of apoptosis by a variety of pharmacological and genetic approaches results in smaller infarction (13, 21, 24), improved cardiac function (4, 18), and attenuated cardiac remodeling (1, 6).

NO is synthesized from L-arginine by three isoforms of NOS, two (inducible NOS and neuronal NOS) of which are constitutively expressed and are acutely regulated by calcium/calmodulin and phosphorylation, whereas the third (iNOS) is induced during inflammation and produces higher levels of NO for a longer period (20, 27). Our present study demonstrated that NO production was not increased until 3 h after trauma, a time point where clear iNOS expression was detected. This result suggests that a newly expressed NOS (i.e., iNOS), rather than phosphorylation activation of eNOS, is responsible for increased NO production after trauma.

NO itself at physiological concentrations is relatively unreactive. However, NO may be converted to a number of more reactive derivatives, known collectively as reactive nitrogen species. In the present study, we have provided direct evidence that O$_2^\cdot$ production is also significantly increased in the traumatized heart, although the molecular sources remain to be determined. Biochemical and in vitro experiments have demonstrated that NO and O$_2^\cdot$ react at the diffusion-limited rate (3 times faster than its detoxification reaction with SOD) (2) to produce peroxynitrite (ONOO$^-\cdot$), a highly reactive species that may oxidize (e.g., cysteine oxidation) or nitrate (e.g., tyrosine nitration) a variety of molecules, thus resulting in cell death and tissue injury via multiple signaling pathway (3, 5, 22, 28).

Moreover, peroxynitrite has been reported to increase apoptotic cell death in a variety of cell types. The proapoptotic mechanisms of ONOO$^-\cdot$ include protein and DNA oxidation (36, 42), lipid peroxidation (34), protein nitration (9, 12, 15, 19, 45), apoptosis-inducing factor releasing (43), and endoplasmic reticulum stress with the subsequent release of caspase 12 (25).

In summary, although numerous studies focusing on the prevention and treatment of mechanical trauma injury that results in immediate organ injury and lethal circulatory shock have been published, we report here the first observation that a mechanical trauma without circulatory shock results in significant cardiomyocyte apoptosis and cardiac dysfunction. Moreover, our results highly suggest that simultaneous generation of NO and O$_2^\cdot$ and subsequent production of ONOO$^-\cdot$ is a mechanism likely responsible for posttrauma myocardial apoptosis, although further studies using a specific ONOO$^-\cdot$ decomposition catalyst may provide a more definitive answer. Nevertheless, these findings are not only scientifically important, but also form the foundation of the search for the optimal therapy to prevent postinjury multiorgan failure because similar mechanisms may apply to other organs.

**REFERENCES**


**GRANT**

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