High-dose Norepinephrine Induces Disruption of Myocardial Extracellular Matrix and Left Ventricular Dilatation and Dysfunction in a Novel Feline Model

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Background: Intravenous norepinephrine (NE) at a dose of 1–6 µg/kg/minute can induce increased extracellular matrix (ECM) and hypertrophic cardiomyopathy. This study aimed to investigate the effects of a higher dose of NE on cardiac remodeling.

Methods: After intraperitoneal urethane-chloralose anesthesia, 7 cats (3.03 ± 0.58 kg) received intravenous infusion of NE 30 µg/kg/minute for 3 hours. Aortic blood pressure and heart rate (HR) were measured by polygraphy at 0, 5, 15, 30, 60, 90, 120, and 180 minutes. Left ventricular size and ejection fraction (EF) were measured by M-mode echocardiography before and after NE administration. Histopathology was performed by hematoxylin-eosin, silver impregnation, and Sirius red staining. Activity of matrix metalloproteinases (MMP) in the left ventricle was measured by zymography.

Results: Mean blood pressure (mmHg) increased from 139 ± 20 to 198 ± 19, 187 ± 23, and 166 ± 16 at 15, 30, and 60 minutes, respectively, during NE infusion. HR (beats/minute) decreased from 214 ± 10 to 158 ± 28 at 15 minutes and then recovered gradually. The left ventricles showed significant dilatation (end-diastolic diameter: from 1.20 ± 0.18 to 1.58 ± 0.23 cm, p = 0.003; end-systolic diameter: from 0.62 ± 0.23 to 1.35 ± 0.29 cm, p = 0.002) and hypokinesia (EF: from 86.2 ± 5.2 to 33.1 ± 16.5%, p < 0.001). Histopathology revealed that left ventricular myocytes were elongated, wavy, and fragmented, while collagen fibers were overstretched, straightened, and disrupted. MMP-9 activity was significantly elevated (p = 0.003 vs. control), while MMP-2 activity was unchanged.

Conclusion: High-dose NE increases MMP-9 activity and causes ECM disruption, left ventricular dilatation and dysfunction. [J Chin Med Assoc 2006;69(8):343–350]

Key Words: extracellular matrix, heart failure, matrix metalloproteinases, norepinephrine

Introduction

Collagens are the major extracellular matrix (ECM) proteins found in the heart.1,2 The fibrillar collagens of the heart surround and interconnect myocytes and muscle fibers to enable muscle fiber and myocyte alignment, which imparts mechanical support to the myocardium and governs tissue stiffness.1,2 It has become increasingly evident that the myocardial ECM is not a static structure but, rather, a dynamic entity that may play a functional role in myocardial adaptation to pathologic stress and thereby facilitate remodeling in different kinds of cardiomyopathy.3–7 Hypertrophic cardiomyopathy is characterized by an
increase in ECM, ventricular thickening, and impaired diastolic function.\textsuperscript{4} However, dilated cardiomyopathy is characterized by a decrease in ECM, ventricular dilatation, and impaired systolic function.\textsuperscript{4–7}

Collagen degradation is an important step in the remodeling of ECM.\textsuperscript{1} Collagens are degraded extracellularly by a family of matrix metalloproteinases (MMPs) capable of enzymatically digesting a wide variety of ECM.\textsuperscript{1,8–17} So far, more than 20 different subtypes of MMP are known.\textsuperscript{8} Among them, matrix gelatinases (MMP-2 and MMP-9) may be involved in the disorganization of the contractile apparatus in dilated cardiomyopathy.\textsuperscript{15} Nishikawa et al\textsuperscript{16} demonstrated that MMP-9 rather than MMP-2 is involved in left ventricular dilatation in systolic heart failure.

Catecholamines are important regulators of myocardial contractility and metabolism.\textsuperscript{18} However, excessive release or administration of catecholamines exceeding physiologic doses may result in reversible or even irreversible cardiac damage.\textsuperscript{18,19} Catecholamine cardiotoxicity or cardiomyopathy is found in patients who have received large amounts of pressor agents and in those with pheochromocytoma or certain brain lesions.\textsuperscript{18–26} Previous animal models of catecholamine cardiomyopathy used relatively lower doses (1–6 µg/kg/minute) and longer (90 minutes–14 days) durations of norepinephrine (NE) administration, which caused hypertrophic remodeling of the ventricle.\textsuperscript{8–10,27–29} However, whether a higher dose of NE treatment has a different effect on the ECM and cardiac remodeling remains unclear. Our present study presents a novel feline model of catecholamine cardiomyopathy using a relatively high dose of NE (30 µg/kg/minute), which can increase MMP-9 activity and cause disruption of the ECM, and left ventricular dilatation and dysfunction within 3 hours.

\textbf{Methods}

\textbf{Animal and experimental procedure}

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Institutional Animal Care and Use Committee of Taichung Veterans General Hospital. After intraperitoneal injection of alpha-chloralose 40 mg/kg and urethane 400 mg/kg for 1 hour, each cat was cannulated in the right femoral artery and vein. The arterial line was connected to a polygraph system for continuous monitoring of blood pressure and heart rate (HR).\textsuperscript{30} NE 30 µg/kg/minute was administered intravenously for 3 hours. M-mode echocardiography (12 MHz probe in Philips Sono 5500, USA) assessed the left ventricular diameter at end-diastole and end-systole and ejection fraction (EF) (Teichholz method provided by the manufacturer) before and after NE treatment.\textsuperscript{31} After completing the experiment, each cat was killed immediately by intravenous potassium chloride injection and the heart was removed. Some of the left ventricular tissues were stored in –70°C liquid nitrogen for the determination of MMP activity, and the others were fixed in 10% buffer formalin for histopathology.

\textbf{MMP activity measured by zymography}

MMP activity was assayed by SDS-PAGE zymography using gelatin as the substrate.\textsuperscript{32} For gelatin-containing zymograms, equal volumes (10 µL) of samples normalized for protein concentration were subjected to electrophoresis, without boiling or reduction, through a 10% polyacrylamide gel with gelatin (0.5 mg/mL). After electrophoresis, the gel was incubated for 1 hour at 25°C in 2.5% Triton X-100 solution, washed twice for 20 minutes each with water and then incubated overnight at 37°C in 0.05 M Tris–HCl buffer, pH 8.0, containing 5 mM CaCl\textsubscript{2}. The gels were fixed with 40% methanol and 7% acetic acid, stained with 0.25% Coomassie brilliant blue R250 and then destained with 10% methanol and 7% acetic acid. Enzyme activity attributed to MMP-2 and MMP-9 is visualized in the gelatin-containing zymograms as clear bands against a blue background. Standards for the active forms of recombinant human MMP-2 and MMP-9 were included in the gels for comparison and identification.

\textbf{Histopathologic examination}

After gross examination of the heart, the left ventricular tissues were fixed in 10% buffer formalin and embedded in paraffin.\textsuperscript{33} The 6 µm sections were stained with hematoxylin-eosin (H-E) and picro-Sirius,\textsuperscript{34} while 25 µm sections were stained with silver impregnation.\textsuperscript{4}

\textbf{Statistical analysis}

All data were expressed as mean ± SD. Repeated measured analysis of variance (ANOVA) test was used for statistical comparisons between the values of mean blood pressure and HR. Paired t test was used for comparison between the values obtained after NE infusion and the baseline value for left ventricular size and EF. Student t test was used for the comparison of MMP activity between NE and control groups. The mean values of the 2 groups were considered significantly different if p < 0.05.
Results

Blood pressure and HR
Figure 1 shows the time course of hemodynamic data. Mean aortic blood pressure increased from 139±20 to 198±19, 187±23, and 166±16 mmHg at 15, 30, and 60 minutes, respectively, during NE infusion ($p<0.001$, $p=0.011$, and $p=0.005$, respectively). It was lower than baseline data at 180 minutes (120±24 mmHg, $p=0.013$) due to heart failure. HR (beats/minute) decreased from 214±10 to 158±28 at 15 minutes and then recovered gradually.

Cardiac size and function
After a 3-hour treatment with NE, the left ventricles showed significant dilatation (end-diastolic diameter: from 1.20±0.18 to 1.58±0.23 cm, $p=0.003$; end-systolic diameter: from 0.62±0.23 to 1.35±0.29 cm,

Figure 1. Time course of mean blood pressure (MBP) and heart rate (HR) during norepinephrine treatment. Values are mean±SD of 7 cats. *$p<0.001$; †$p<0.05$; ‡$p<0.01$ vs. baseline values.

Figure 2. Two-dimensional echocardiography in the short-axis view shows significant dilatation of the left ventricle. (A) Before and (B) after electrical stimulation.
$p = 0.002$) concomitant with hypokinesia (EF: from $86.2 \pm 5.2\%$ to $33.1 \pm 16.5\%$, $p < 0.001$) (Figures 2 and 3).

**MMP activity**

A representative zymogram for left ventricular MMP activity is shown in Figure 4A. Clear proteolytic bands were revealed on gelatin zymography gels corresponding to both gelatinase MMP-9 and MMP-2. Quantitative analysis demonstrated that the increase in MMP-9 zymographic activity was more pronounced in the norepinephrine (NE) group ($n = 7$) than in the control group ($n = 6$) ($p = 0.003$). There was no difference in MMP-2 zymographic activity between NE and control groups ($p = 0.192$). *$p < 0.01$ vs. control.

**Histopathologic findings**

Gross examination of NE-treated hearts revealed remarkable dilatation of both ventricles indicated by rounding of the apex (Figure 5A). Myocardial hemorrhage was also noted, especially at the base of the papillary muscles (Figures 5A and B). In H-E stain, cardiomyocyte damage was characterized by myofibrillar weaving (Figure 5C), elongation, and disruption (myocytolysis, Figure 5D). Normal collagens of...
myocardial ECM are perimysial coil-like fibers, which are parallel to the cardiomyocytes in picro-Sirius (Figures 6A and C) and silver impregnation staining (Figure 6E). In NE-treated hearts, they became over-stretched, progressively straightened, and ultimately disrupted (Figures 6B, D and F).

Discussion

The main finding in this study was that a high dose of NE of 30 µg/kg/minute could increase MMP-9 but not MMP-2 expression, which was associated with the disruption of ECM and left ventricular dilatation and dysfunction. MMPs are responsible for ECM degradation and remodeling, so previous studies concluded that the activation of MMPs plays a crucial role in left ventricular dilatation, and MMPs are therapeutic targets for the prevention of left ventricular remodeling.\textsuperscript{5,11,16} However, whether all activated MMPs contribute equally to left ventricular remodeling is not known. Nishikawa et al\textsuperscript{16} demonstrated that activation of MMP-2 occurred in association with progressive myocardial fibrosis and was independent of ventricular dilatation. In contrast, although MMP-9 was somewhat activated in diastolic heart failure, its activity was more enhanced in systolic heart failure with left ventricular dilatation.\textsuperscript{16} Moreover, immunohistochemical study and \textit{in situ} zymography demonstrated that the region with increased gelatin lysis corresponded to that with increased MMP-9 expression.\textsuperscript{16} The above findings suggest that MMP-9 is likely to contribute to left ventricular dilatation irrespective of underlying cardiovascular diseases. Li et al\textsuperscript{17} reported downregulation of MMP-9 in patients with dilated cardiomyopathy following support with left ventricular assist devices. This suggests that an increased left ventricular filling pressure and, hence, an elevated

\textbf{Figure 5.} Gross examination of norepinephrine-induced hearts showed: (A) remarkable dilatation of both ventricles indicated by rounding of the apex; (B) myocardial hemorrhage was also noted, especially at the base of the papillary muscles. In hematoxylin-eosin stain, cardiomyocyte damage was characterized by: (C) myofibrillar weaving and (D) disruption (myocytolysis).
left ventricular wall stress are responsible for the enhanced expression of MMP-9. Our results showed that a high dose of NE of 30 µg/kg/minute could increase MMP-9 expression, which resulted in the disruption of ECM and left ventricular dilatation and dysfunction, whereas MMP-2 was unchanged. This cardiac remodeling is different from that induced by a lower dose of NE. Previous studies demonstrated that

Figure 6. Normal collagens of myocardial extracellular matrix are coil-like fibers, which are parallel to the cardiomyocytes in picro-Sirius (A, C) and silver impregnation staining (E). In norepinephrine-treated hearts, they became overstretched, progressively straightened, and ultimately disrupted (B, D, F).
NE at a dose of 1–6 µg/kg/minute induced hypertrophic remodeling of the heart, especially the left ventricle. These findings indicate that a differential remodeling response to NE is dose-dependent. A low dose of NE increases MMP-2 expression, which is a sign of the remodeling process accompanied by cardiac fibrosis. However, a high dose of NE increases MMP-9 expression, which facilitates disruption of ECM and results in ventricular dilatation.

In our model, an increase in blood pressure occurred abruptly after NE administration and persisted for about 60 minutes (Figure 1). However, it was initially accompanied by a decrease and not an increase in HR. There are some reasons for this. First, hypertension can activate baroreflex and parasympathetic activity. Second, high-dose NE can damage not only the myocytes and ECM, but also the pacemaker cells in the heart. We observed arrhythmia, including ventricular premature beat, sinus bradycardia and sinus arrhythmia in the initial 30 minutes. After that, HR recovered at 60 minutes and became elevated at 120 minutes. The latter tachycardia might be due to sympathetic compensation because of heart failure.

Many studies have revealed that the myocardial damage in catecholamine cardiotoxicity is coagulative myocytolysis, also known as myofibrillar degeneration or contraction band necrosis, which is characterized by sarcoplasmic coagulation, granularity, vacuolization, and myofibrillar disruption. In addition to its well-known effect on myocytes, NE can alter the ECM and regulate cardiac remodeling. Our observations showed that the deformed collagen fibers in the ECM caused by the huge dose of NE contributed to the dilatation and dysfunction of the left ventricle.

In conclusion, an extremely high dose of NE increases MMP-9 activity and causes disruption of the ECM and left ventricular dilatation and dysfunction. The inhibition of MMP-9 activity might be a therapeutic target for the prevention of NE-induced cardiac remodeling.

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References


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