NOTE *Toxicology*

Aberrant gene expression of heparanase in ventricular hypertrophy induced by monocrotaline in rats

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ABSTRACT. The gene expression levels of heparanase, matrix metalloproteinase 2 (MMP2) and MMP9 were examined in ventricles after treatment with monocrotaline (MCT) to induce cardiac hypertrophy in rats. Rats received a single intraperitoneal injection of MCT (60 mg/kg) or saline. Twenty-five days after the injection, the right ventricle and lung wet weights were increased in MCT-treated rats compared with the control. Histological analysis revealed cardiomyocyte hypertrophy in the right ventricle of MCT-treated rats. Northern blot hybridization showed that heparanase and MMP2 expression increased significantly in the right and left ventricles of MCT-treated rats, whereas MMP9 was not induced. These findings indicate that heparanase and MMP2 might play an important role in the development of MCT-induced cardiac hypertrophy.

KEY WORDS: cardiac hypertrophy, heparanase, matrix metalloproteinase, monocrotaline

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Heparanase and matrix metalloproteinases (MMPs) are extracellular matrix (ECM)-degrading enzymes that degrade the heparan sulfate side chain of heparan sulfate proteoglycan and type IV collagen, respectively, in the ECM [2, 10]. Remodeling of the ECM contributes to various pathological conditions, such as inflammation, tumor angiogenesis and metastasis, cardiac hypertrophy and congestive heart failure [5, 7, 16]. MMPs have an important role in the development of clinical and experimental cardiac diseases models through degradation of the ECM [10, 24]. However, little is known regarding the role of heparanase on cardiac function and the development of cardiac diseases.

Right ventricular hypertrophy is a general adaptive mechanism of the heart to increased workload resulting from chronic pulmonary hypertension, vascular disease or left ventricular dysfunction [4]. Administration of a pyrrolizidine alkaloid, monocrotaline (MCT), to rats is used as a noninvasive, slowly developing, hemodynamically relevant model of pulmonary hypertension leading to right ventricular hypertrophy and failure [9].

It has been reported that MMP2 and MMP9 expressions were increased in right ventricular hypertrophy induced by MCT treatment in rats [26] and that captopril attenuates the expression of MMPs and the development of MCT-induced right ventricular hypertrophy [19, 20]. However, the changes in myocardial heparanase expression after treatment with MCT have not been studied. The present study was undertaken to address this after treatment of rats with MCT.

Male Wistar rats (six weeks old, CLEA Japan, Tokyo, Japan) were housed in standard cages, maintained on a standard laboratory diet and tap water and exposed to a 12 hr light-dark cycle. The ambient temperature was maintained at about 23°C during the study. All animals were cared for in accordance with the guidelines for animal treatment of Kitasato University, which meet international guiding principles of laboratory animal care. The animal experiment was carried out at Kitasato University. Right ventricular hypertrophy was induced with MCT as described by Seyfarth *et al.* [23]. Rats were randomly selected to receive either an intraperitoneal injection of MCT (60 mg/kg body weight, Wako Pure Chemical Industries, Osaka, Japan) or an equal volume of physiological saline (2.5 m*l*/kg body weight). MCT was dissolved in 1 M HCl, neutralized with 1 M NaOH, diluted with physiological saline and then injected at a concentration of 24 mg/m*l*. At 25 days after MCT injection, hearts and lungs were excised under sodium pentobarbital (50 mg/kg, i.p.) anesthesia. The hearts were divided into the right ventricle (RV) and left ventricle including the intraventricular septum (LV). These tissues were frozen quickly in liquid nitrogen and stored at −80°C until use for RNA extraction. The excised hearts were fixed in 10% neutral formalin. The

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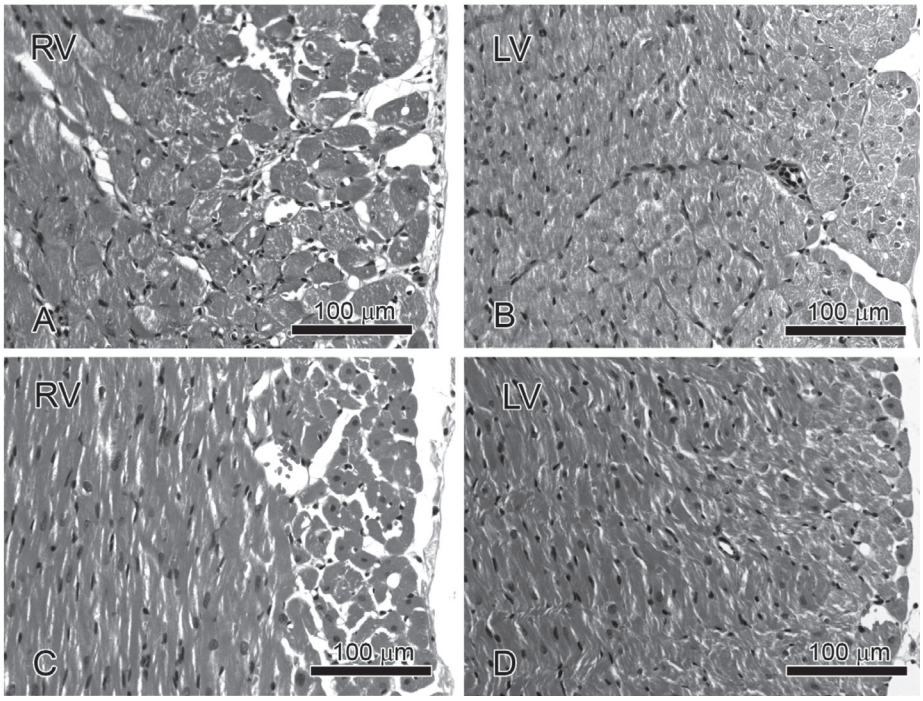

Fig. 1. Photomicrographs of ventricular tissue sections obtained from control and MCT-treated rats. Representative photomicrographs of HE-stained sections of right (A and C) and left (B and D) ventricles treated with (A and B) or without (C and D) MCT. Scale bars represent 100 μm.

tissues were dehydrated and embedded in paraffin wax. For histological analysis, tissue sections (4–5 μm) were stained with hematoxylin and eosin (HE) as described previously [28].

Total RNA was isolated from frozen ventricular tissues using ISOGEN (Nippon Gene, Toyama, Japan). DIG-labeled cRNA antisense and sense probes for heparanase, MMP-2 and MMP-9 were prepared; detection of rat heparanase, MMP2 or MMP9 mRNA with these cRNA probes has been described previously [13]. In brief, 5 μg of total RNA was fractionated on a 1.2% agarose–formaldehyde gel and transferred onto a positively charged nylon filter. Prehybridization, hybridization and signal detection were performed as described previously [12].

All data are presented as the mean and SEM, and they were analyzed statistically using the JMP software (SAS Institute, Cary, NC, U.S.A.) with the Student's *t*-test or one-way analysis of variance followed by a Tukey-Kramer multiple comparison test. For all data, $P<0.05$ was considered significant.

Table 1 shows the biometric changes of the animals at 25 days after MCT treatment. Body weights decreased significantly in the MCT-treated rats compared with the control ($P<0.05$). Right ventricular weight was corrected by tail length, and the right ventricular weight/tail length ratio increased by about 2-fold in the MCT-treated rats compared with the control ($P<0.05$). Histological differences between control and MCT-treated rats are illustrated in Fig. 1. Microscopic examination identified marked cardiomyocyte hypertrophy in the right ventricle of MCT-treated rats in contrast to control (Fig. 1A and 1C). Although the sum of left ventricle and interventricular septum weight/tail length ratio was not different between the control and MCT-treated rats, the HE staining of the left ventricle section revealed that weakly hypertrophied myocytes were present in MCT-treated rats (Fig. 1B and 1D). Lung wet weight and the weight/tail length ratio increased in the MCT-treated rats compared with the control ($P<0.05$) (Table 1).

Table 1. MCT-induced changes in various parameters in rats

Parameters	Control (n=4)	MCT (n=5)
Body weight (g)	323 ± 4	261 ± 7*
Tail length (cm)	16.7 ± 0.3	15.6 ± 0.3
RV weight (mg)	170 ± 5	339 ± 23*
LV weight (mg)	724 ± 12	700 ± 20
Lung wet weight (mg)	1318 ± 70	2070 ± 144*
RV weight /tail length ratio (mg/cm)	10.4 ± 0.4	21.8 ± 1.3*
LV weight/tail length ratio (mg/cm)	44.0 ± 1.5	45.0 ± 1.0
Lung wet weight/tail length ratio (mg/cm)	79.1 ± 5.7	133.7 ± 11.1*

Data are presented as the mean ± SEM. RV: Right ventricle. LV: Left ventricle including interventricular septum. *$P<0.05$: compared with control group.

Northern blot hybridization was used to investigate heparanase, MMP2 and MMP9 expression in the right and left ventricles. Heparanase mRNA was slightly expressed in

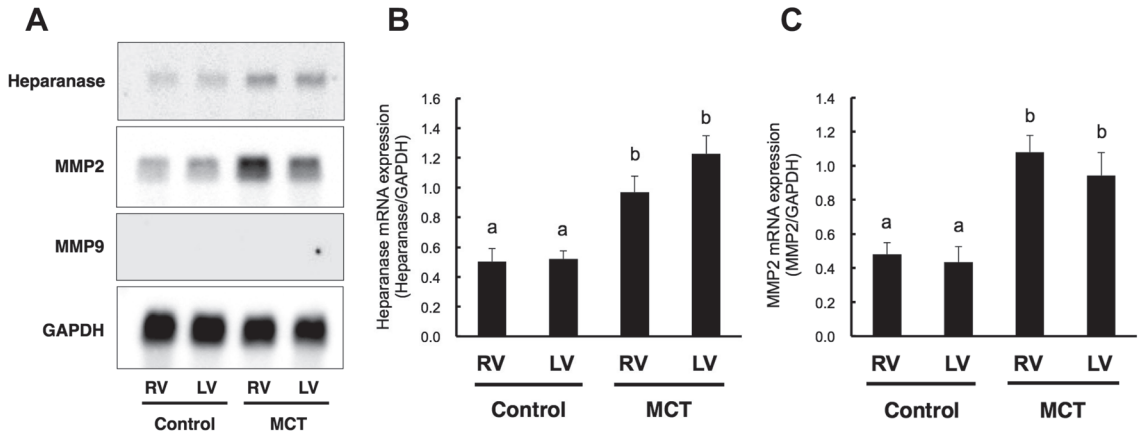

Fig. 2. Expression of heparanase, MMP2 and MMP9 mRNA in right and left ventricles of MCT-treated rats. Representative Northern blot image of heparanase, MMP2, MMP9 and GAPDH in the right (RV) and left (LV) ventricles of control and MCT-treated rats (A). Densitometric analyses of heparanase (B) and MMP2 (C) mRNA levels. Levels were normalized to GAPDH. Data are given as the mean ± SEM (n=4–5). Data labeled with different letters are significantly different from each other ($P<0.05$).

the right and left ventricles in control rats (Fig. 2A and 2B). In MCT-treated rats, the mRNA expression was increased significantly, by approximately 2-fold, in both ventricles at 25 days after treatment. MMP2 mRNA was also detected in both ventricles of control rats and was increased in the MCT-treated rats by as much as the heparanase expression (Fig. 2A and 2C). On the other hand, expression of MMP9 mRNA was not detected in the ventricles of the control and MCT-treated rats (Fig. 2A). The cRNA probe for MMP9 detected rat MMP9 in lung tissue as a positive control (data not shown).

The present study demonstrated that the gene expression of heparanase was induced in the rat myocardium after treatment with MCT. Enhanced expression of heparanase has been reported in various pathological conditions including cancer [2]. Although we have reported previously the induction of heparanase expression in the ventricular myocardium of rats with isoproterenol-induced left ventricular hypertrophy [13], the changes in the expression of heparanase in cardiac disease are not still clear. Therefore, we examined that gene expression of heparanase in right ventricular hypertrophy following treatment with MCT.

Administration of MCT to rats has been used as a model of pharmacologically and toxicologically induced pulmonary hypertension leading to right ventricular hypertrophy and failure [8, 9, 20]. Right ventricular hypotrophy induced by a single intraperitoneal injection of 60 mg/kg MCT was confirmed by the increases in right ventricular weight/tail length ratio and lung wet weight, which were consistent with previous reports [8, 9, 20]. Furthermore, histological examination showed that treatment of rats with MCT caused marked cardiomyocyte hypertrophy in the right ventricle. These results indicate that a single intraperitoneal injection of MCT caused right ventricular hypertrophy in rats in the present study.

It is well known that remodeling of the myocardial ECM is critical for development of cardiac diseases [3, 10]. In the present study, heparanase and MMP2 expressions were increased significantly in MCT-treated rats. Heparanase and MMP2 degrade the components of the ECM including the heparan sulfate side chain of heparan sulfate proteoglycan and type IV collagen and may play roles in the process of ventricular remodeling in the hypertrophied heart caused by MCT.

The ECM acts as not only a space-filling material but also as storage for bioactive molecules, which modulate cell adhesion, migration, proliferation, differentiation and survival [11]. It has been reported that epidermal growth factor receptor (EGFR) activation by heparin-binding EGF-like growth factor (HBEGF) plays an important role in the induction of cardiac hypertrophy [1]. In addition, HBEGF induces a hypertrophic response in rat cardiomyocytes, which suggests that it acts as an autocrine hypertrophic factor [21].

HBEGF shedding on the cell surface of cardiomyocytes resulting from metalloproteinases activation and subsequent activation of EGFR occurs when the cells are stimulated by G-protein-coupled receptor agonists, such as adrenergic agonist, angiotensin II and thrombin [1, 25]. HBEGF binds to the heparan sulfate side chain of heparan sulfate proteoglycan in the ECM and is released and activated when heparanase degrades heparan sulfate [2]. In the myocardial ECM, heparanase-mediated release of HBEGF might be necessary to elicit transactivation of EGFR in cardiomyocytes. In addition to heparanase expression, we observed enhanced expression of MMP2 in ventricles of MCT-treated rats. We have reported previously a basically similar result using an isoproterenol-induced cardiac hypertrophy model [13]. Therefore, our findings suggest that MMP2 contributes to the process for shedding of HBEGF.

Interestingly, we found enhanced expression of heparanase and MMP2 not only in the right ventricle, but also in the left ventricle, which was weak or not hypertrophied. It has

been reported that right ventricular hypertrophy induced by MCT causes impairment of left ventricular diastolic function due to structural changes of the RV and LV in rats [14]. Furthermore, Lourenço *et al.* reported that the left ventricular myocardium is altered in advanced MCT-induced right ventricular hypertrophy undergoing neurohumoral activation [15]. Therefore, these findings indicate that heparanase and MMP2 may also contribute to ECM remodeling or the function of cardiomyocytes in the left ventricle in the MCT-induced right ventricular hypertrophy model. Although heparanase and MMP2 were induced in the hypertrophy models, we could not ascertain whether cardiac hypertrophy is induced directly by these factors. Further studies on time-dependent changes in expression and the effect of specific inhibitors in the MCT-induced cardiac hypertrophy model are needed.

The precise molecular mechanism underlying induction of gene expression of heparanase and MMP2 is not clear in the present study. Transcription of the human heparanase gene is increased by activation of the transcription factor early growth response-1 (EGR1) [17, 18]. Furthermore, EGR1 contributes to isoproterenol and MCT-induced cardiac hypertrophy [6, 22]. Therefore, myocardial heparanase induction might be mediated by increased expression of EGR1 in MCT-induced cardiac hypertrophy. On the other hand, expression of MMPs is stimulated by numerous factors including angiotensin II [29], and the neurohumoral factor is also elevated in MCT-induced cardiac hypertrophy [27]. These reports indicate that angiotensin II modulates MMP2 expression in cardiac hypertrophy induced by MCT treatment.

The present study does not directly address the localization and protein expression of heparanase and MMP2 in the myocardium. Further studies on localization, protein expression and activities of these factors in the myocardium are required for better understanding of the role of heparanase and MMP2 in development of MCT-induced cardiac hypertrophy.

In conclusion, we demonstrated that the respective expressions of the genes for heparanase and MMP2 were increased in the right and left ventricles after treatment with MCT in rats. However, the gene expression of MMP9 was not induced in both ventricles. Although a further study is required, our results suggest that heparanase and MMP2 might serve an important role in the development of MCT-induced cardiac hypertrophy.

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