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Dynamic Induction of ADAMTS1 Gene in the Early Phase of Acute Myocardial Infarction

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Extracellular matrix (ECM)–degrading enzymes such as matrix metalloproteases (MMPs) play an essential role in the repair of infarcted tissue, which affects ventricular remodeling after myocardial infarction. ADAMTS1 (A disintegrin and metalloprotease with thrombospondin motifs), a newly discovered metalloprotease, was originally cloned from a cancer cell line, but little is known about its contribution to disease. To test the hypothesis that ADAMTS1 appears in infarcted myocardial tissue, we examined ADAMTS1 mRNA expression in a rat myocardial infarction model by Northern blotting, real-time RT-PCR and *in situ* hybridization. Normal endothelium expressed little ADAMTS1 mRNA, while normal myocardium expressed no detectable ADAMTS1 mRNA. Up-regulation of ADAMTS1 was demonstrated by Northern blot analysis and real-time RT-PCR at 3 h after coronary artery ligation. *In situ* hybridization revealed strong ADAMTS1 mRNA signals in the endothelium and myocardium in the infarcted heart, mainly in the infarct zone, at 3 h after myocardial infarction. The rapid and transient up-regulation of the ADAMTS1 gene in the ischemic heart was distinct from the regulatory patterns of other MMPs. Our study demonstrated that the ADAMTS1 gene is a new early immediate gene expressed in the ischemic endothelium and myocardium.

Key words: ADAMTS, coronary artery disease, extracellular matrix, ischemia, matrix metalloprotease.

Acute myocardial infarction (MI) is a leading cause of death and disability in the Western world. The extracellular matrix (ECM) in the myocardium has a role in pathogenesis in the healing process after MI (*1–3*). For example, left ventricular (LV) enlargement frequently develops after MI. Myocardial loss as a consequence of infarction initiates a vicious cycle of contractile dysfunction and progressive LV dilation, referred to as ventricular remodeling. Ventricular remodeling is associated with heart failure and increased mortality (*4*, *5*). Dynamic changes between the accumulation and degradation of ECM molecules are involved in the process of ventricular remodeling, and ECM homeostasis regulates this process to preserve LV myocyte alignment, force transmission, and overall contraction and relaxation (*6–8*). Many biological substances, including proteases and their inhibitors and growth factors, contribute to this ECM reformation through interactions with the components of the ECM (*9–11*). In particular, the increased expression and activation of matrix metalloproteinases (MMPs) have been implicated in this process (*12*, *13*). The importance of MMPs in MI and LV remodeling has been demonstrated, and the expression, distribution and functional role of MMPs in MI have been examined (*14*, *15*). Recent reports showed that MMP-inhibition reduces LV dilation and preserves LV ejection fraction in animal heart failure models (*16–18*).

Ventricular remodeling is also affected by the infarct size, which can be limited by opening the infarct-related artery (*i.e.*, reperfusion and arterial patency) or by the formation and development of collateral vessels (*19*). The development of collateral vessels, termed angiogenesis after MI, has beneficial effects on the infarct healing, and angiogenic growth factors are considered to have roles in MI. Recently, a number of experimental studies have suggested that treatment with angiogenic growth factors can promote the development of collaterals in animal MI models (*20*, *21*). Vascular endothelial growth factor (VEGF) is an endothelial cell mitogen that is thought to function in angiogenesis. VEGF is increased as early as 1 h after coronary artery ligation (*22*).

The gene for ADAMTS1 (A disintegrin and metalloprotease with thrombospondin motifs-1), a newly discovered metalloprotease, was initially cloned as an inflammatory response-associated gene (*23*). ADAMTS1 is not expressed in normal tissues but is induced by lipopolysaccharide (LPS) stimulation. A recent study demonstrated that ADAMTS1 and ADAMTS4 have key roles in versican proteolysis (*24*). Compared with MMPs, ADAMTS proteases recognize a more diverse range of substrates, such as procollagens and proteoglycans (*24*, *25*). ADAMTS1 was also reported to have another func-

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tion as an angiogenesis inhibitor (*26*). ADAMTS1 protein suppressed fibroblast growth factor-2–induced vascularization in the cornea pocket assay and inhibited VEGF-induced angiogenesis in the chorioallantoic membrane assay (*26*). ADAMTS1 was recently reported to bind to VEGF and abrogate the phosphorylation of its receptor, VEGFR2 (*27*, *28*). These previous studies led us to hypothesize that ADAMTS1 is involved in MI. However, there have been no studies examining the contribution of ADAMTS1 to cardiovascular disease. Accordingly, we examined the ADAMTS1 expression and its distribution in experimentally induced MI in rats.

MATERIALS AND METHODS

Experimental Acute Myocardial Infarction—All protocols involving experimental animals followed the local institutional guidelines for animal care, which are comparable to "Guide for the Care and Use of Laboratory Animals" published by the Institute for Laboratory Animal Research (National Institutes of Health publication No. 85-23, revised 1996). The method for induction of MI has been reported by us as well as elsewhere (*29*, *30*). Briefly, adult Sprague-Dawley male rats weighing 200 to 250 g were purchased. The rats were anesthetized and the left coronary artery was ligated. The induction of MI was confirmed by a cardiac surface color change from reddish to a pale color and by ST-segment elevation documented by continuous electrocardiographic monitoring or the appearance of ventricular arrhythmia. Echocardiographic studies were performed before the surgical procedure and immediately before sacrificing animals as previously described (*31*). LV wall motion asynergy due to MI was confirmed using commercially available equipment (ProSound SSD-4000; Aloka Co., Ltd., Tokyo, Japan).

For Northern blot analysis, rats were sacrificed at 3 h, 6 h, 12 h, 1 d, 2 d, 7 d, 14 d, and 28 d post-MI (n = 6 at each time point). In each analysis, sham-operated rats were also used at the indicated time points to serve as a control group. For the *in situ* hybridization, 3 rats were sacrificed in each group 3 h after ligation and in a control group.

RNA Extraction and cDNA Synthesis—The non-infarct zone was removed from the excised heart. Infarct zone containing the infarct border zone was snap-frozen in liquid N_2, pulverized, and resuspended in RNAzolB (Tel-Test., Friendswood, TX, USA), and then RNA was extracted using guanidine isothiocyanate according to the manufacturer's protocol. Residual DNA was removed by treatment with 5 units of DNase I (Roche Diagnostics Ltd, Lewes, UK) at 37°C for 45 min followed by inactivation at 65°C for 10 min. Two micrograms of RNA was reverse-transcribed with Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The cDNA was diluted five-fold prior to PCR amplification.

Anti-ADAMTS-1 Antibody—Primary antibody for ADAMTS-1 (A-19) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) and used at a 1: 100 dilution. The cross-reactivity was confirmed before the study to agree with that described on the manufacturer's data sheet. We also raised a polyclonal antibody against ADAMTS-1. A peptide with the sequence CNWQKQHNPPSDRDAEHYD (residue numbers 316–334 in human ADAMTS-1) was synthesized, conjugated to keyhole limpet hemocyanin, and incubated with *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester. A rabbit was immunized with the conjugated peptide by conventional methods, and antiserum was obtained as we previously reported (*32*, *33*). The antibody was affinity purified using this synthetic peptide.

Northern Blotting—The 451-bp cDNA fragment corresponding to nt 183–633 of rat ADAMTS1 cDNA (accession no. NM_024400) was generated by RT-PCR and ligated into the Topo TA cloning vector (Invitrogen). The primers for probe preparation were: sense primer, 5′-TAACTCGGTGCTGGAATAAA-3′ (identical to nt 183–202 of rat ADAMTS1 cDNA); antisense primer, 5′-AGGAGTGTGGTGGAATCGTG-3′ (complementary to nt 615–633 of rat ADAMTS1 cDNA) (*30*, *34*).

As we previously reported, aliquots of total RNA (20 μg) were electrophoresed and transferred to nylon membranes (*30*, *32*). After ultraviolet cross-linking, the filters were prehybridized for 1 h at 65°C, then hybridized with α^{32}P-labelled cDNA probes at 65°C for 3 h. The radiolabelled filters were then washed under stringent conditions, exposed to an imaging plate (Fuji Photo Film Inc., Tokyo, Japan) and developed using an image-analyzing system (BAS-2000, Fuji Photo Film Inc.). Densities of the hybridized bands for ADAMTS1 were quantified densitometrically using an image-analysis program with a computer and standardized relative to the density of the 28S rRNA bands.

Quantitative Real-Time RT-PCR—The mRNA expression of ADAMTS1, α2(I) collagen, VEGF and GAPDH in MI was analyzed in more detail by a quantitative real-time RT-PCR method using a LightCycler rapid thermal cycler system (Roche Diagnostics Ltd.) according to the protocol recently reported for MI rats (*35*). For the real-time PCR analysis, 88 rats were sacrificed in groups of 6 at 3, 6, and 12 h, and 1, 2, 7, 14, and 28 d post-surgery, and in groups of 5 rats with sham-operated hearts at each of these times. To validate the amplification specificity of ADAMTS1, α2(I)collagen, VEGF and GAPDH, we analyzed each PCR product by agarose gel electrophoresis after real-time detection. In addition, we analyzed the melting curve of each PCR product in each PCR session and confirmed that no non-specific products had been produced. There was rarely significant primer dimer formation during the numbers of cycles required for quantification of the PCR products from a range of experimental samples. Each RT-PCR was repeated at least three times to confirm reproducibility. Negative controls were checked with samples in which the RNA templates were replaced by nuclease-free water in the reactions. The PCR primers used in this study are listed in Table 1. Data were analyzed using the absolute standard curve method, as described (*35*). The intra- and interassay coefficients of variation were <5% and were reasonably small compared with those in another report (*35*). The amplification of a housekeeping gene, GAPDH, was used for normalizing the efficiencies of cDNA synthesis and the amount of RNA applied (*36*). The copy number of GAPDH in each sample was calculated by comparison to the known amount of template used as a positive control for each PCR, resulting in a correction factor for each sample. The scores of "relative ADAMTS1," which indicates

Table 1. **The primers for the quantitative real-time RT-PCR analysis were listed.**

ADAMTS1	
Upper	5′-CTCCGGTGGCTTACTGGTGT-3′
Lower	5′-TGTTTTTCCGTTATTGTCTG-3′
α2(I) collagen	
Upper	5′-TACAACGCAGAAGGGGTGTC-3′
Lower	5′-CCTCAGCAACAAGTTCGACG-3′
VEGF	
Upper	5′-TCTTCAAGCCATCCTGTGT-3′
Lower	5′-CTTTCTTTGGTCTGCATTC-3′
GAPDH	
Upper	5′-AACACAGTCCATGCCATCAC-3′
Lower	5′-TCCACCACCCTGTTGCTGTA-3′

the expression of ADAMTS1 relative to that GAPDH, were obtained by dividing the copy number of ADAMTS1 in a sample by the corresponding correction factor.

In Situ Hybridization—*In situ* hybridization was carried out following the protocol we previously described (*30*, *37*). Paraffin-embedded 5-μm sections were used. The same 451-bp cDNA fragment as used in the Northern blot analysis, corresponding to nt 183–633 of rat ADAMTS1 cDNA, was used for *in situ* hybridization. Anti-sense and sense digoxigenin-UTP–labelled cRNA probes were synthesized by *in vitro* transcription with the relevant RNA polymerases (Roche Diagnostics Ltd.). Hybridization was carried out overnight at 42°C in a humidified chamber with digoxigenin-UTP–labelled anti-sense or sense probe for ADAMTS1. The immunological detection of digoxigenin-labelled transcripts was performed according to the manufacturer's protocol (Boehringer-Mannheim). Finally, the sections were lightly counterstained with Mayer's hematoxylin and mounted with Crystal mount (Biomeda, Foster, CA, USA). The level of mRNA signal was graded as follows: –, not observed; ±, positive but faint; +, slight but apparent; ++, moderate; and +++, maximal. The inter-observer reliability and intra-observer reliability were assessed using 50 sample photographs for each grade before the grading analysis. Two investigators conducted each assessment in a blind manner; when the two investigators disagreed, an additional blind observer also made an assessment.

Western Blotting—Western blotting was performed following the protocol we previously described (*38*). Briefly, proteins were extracted from the hearts using CelLytic™ MT (Sigma, St. Louis, MO, USA). Protease inhibitor cocktail (Sigma) was added just prior to the addition of lysis buffer to the tissue samples. Protein concentrations were measured using the DC protein assay (Bio-Rad, Hercules, CA, USA). From each extract, approximately 80 μg of total protein was separated on a 5–20% sodium dodecyl sulfate (SDS)–polyacrylamide gradient gel with a 4% stacking gel (Ready Gels J™, Bio-Rad). After electrophoresis, proteins were transferred to a PVDF membrane (Bio-Rad) using transfer buffer that contained 25 mM Tris-HCl and 200 mM glycine. The membrane was blocked in 5% milk dissolved in 1X TTBS buffer (14 mM Tris-HCl, pH 7.5, 154 mM NaCl, and 0.5% Tween-20) overnight at 4°C. The primary antibodies were incubated for 2 h at room temperature (RT) in 5% milk dissolved in 1XTTBS. The membrane was washed in 1X TTBS three

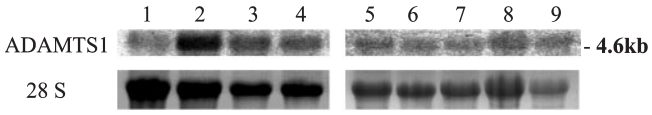

Fig. 1. **Northern blot analysis for ADAMTS1 mRNA in an infarct heart.** Lane 1, control (preligation heart); lane 2, infarct zone at 3 h; lane 3, infarct zone at 6 h; lane 4, infarct zone at 12 h; lane 5, control (preligation heart); lane 6, infarct zone at 2 d; lane 7, infarct zone at 7 d; lane 8, infarct zone at 14 d; lane 9, infarct zone at 28 d. 28S rRNA bands stained with ethidium bromide are shown below.

times for 15 min each at RT. Appropriate secondary antibodies conjugated to horseradish peroxidase (Promega) were incubated with membranes for 1 h at RT. Following five successive washes with 1× TTBS, the membrane was developed using an ECL+ kit (Amersham Pharmacia Biotech). All images were processed for publication using Adobe Photoshop software.

Statistical Analysis—All data are expressed as the mean value ± SE. Statistical comparisons of means were performed by analysis of variance (ANOVA) followed by the Tukey Kramer test. A p value of <0.05 was considered statistically significant.

RESULTS

Northern Blotting and Quantitative Real-Time RT-PCR Analysis—Figure 1 shows the bands detected in MI by Northern blot analysis. A positively hybridized band at 4.6 kb was clearly observed as a single band, which matches the findings previously reported (*26*). ADAMTS1 mRNA was induced 3 h after induction of MI. At later stages (2, 7, 14, or 28 d after MI), ADAMTS1 mRNA was not strongly expressed as compared to the normal heart.

To examine the change in the level of ADAMTS1 mRNA in the early phase of MI, samples at each time point were analyzed by quantitative real-time RT-PCR. Quantitative real-time RT-PCR technology was employed because it allows more samples to be analyzed at once than Northern blot analysis. For quantitative analysis, we used GAPDH as the internal control, as reported elsewhere for the rat MI model (*39*, *40*). Figure 2A shows the time-dependent change in ADAMTS1 mRNA expression determined by quantitative real-time RT-PCR. At 3, 6, 12, and 24 h after MI, the relative expression level of ADAMTS1 mRNA in the infarct zone was, respectively, 4.4-, 5.2-, 3.1-, 2.6-fold greater than that in 3-h sham–operated hearts. At 2, 7, 14, and 28 d after MI, the relative expression level of ADAMTS1 mRNA was, respectively, 1.8-, 2.0-, 1.8-, and 1.0-fold greater than that in 3-h sham–operated hearts. In contrast, the relative α2(I) collagen mRNA levels in the infarct zone at 3 h , 6 h, 12 h, 24 h and 2 d, 7 d, 14 d and 28 d were 0.6-, 0.7-, 0.3-, 0.8-, and 6.7- , 18.3-, 10.2 and 10.0-fold, respectively, relative to those in 3-h sham–operated hearts (Fig. 2B). The relative level of α2(I) collagen was significantly increased in the infarcted hearts 2 d, 7 d, 14 d and 28 d after MI compared with the level in sham-operated hearts ($p < 0.05$). Figure 2C shows the time course of the alteration of VEGF mRNA expression in the infarcted heart. The relative level of VEGF mRNA was significantly increased at

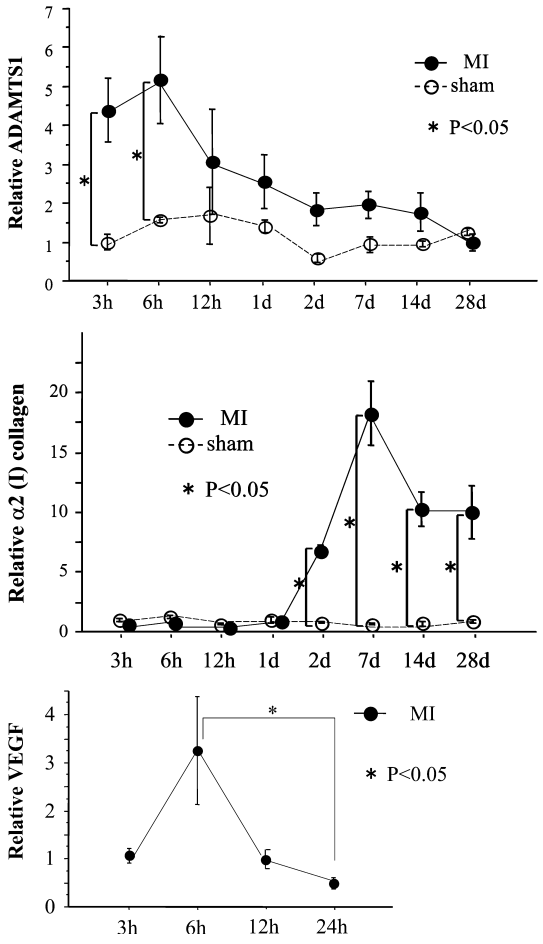

Fig. 2. **A: Quantitative real-time RT-PCR analysis of ADAMTS1 mRNA in the infarcted hearts.** Note that ADAMTS1 mRNA was increased as early as 3 h, and the transient induction of ADAMTS1 reached its peak 6 h after coronary artery ligation. **B: Quantitative real-time RT-PCR analysis of α2(I) collagen mRNA in the infarcted hearts.** α2(I) collagen increased gradually and reached its peak at 7 d. **C: Quantitative real-time RT-PCR of VEGF mRNA in the infarcted hearts.** In the infarct zone, VEGF reached its peak 6 h after ligation, exhibiting a temporal pattern similar to that of ADAMTS1 mRNA. All the quantitative real-time PCR analyses were repeated three times. A p value of <0.05 was considered significant.

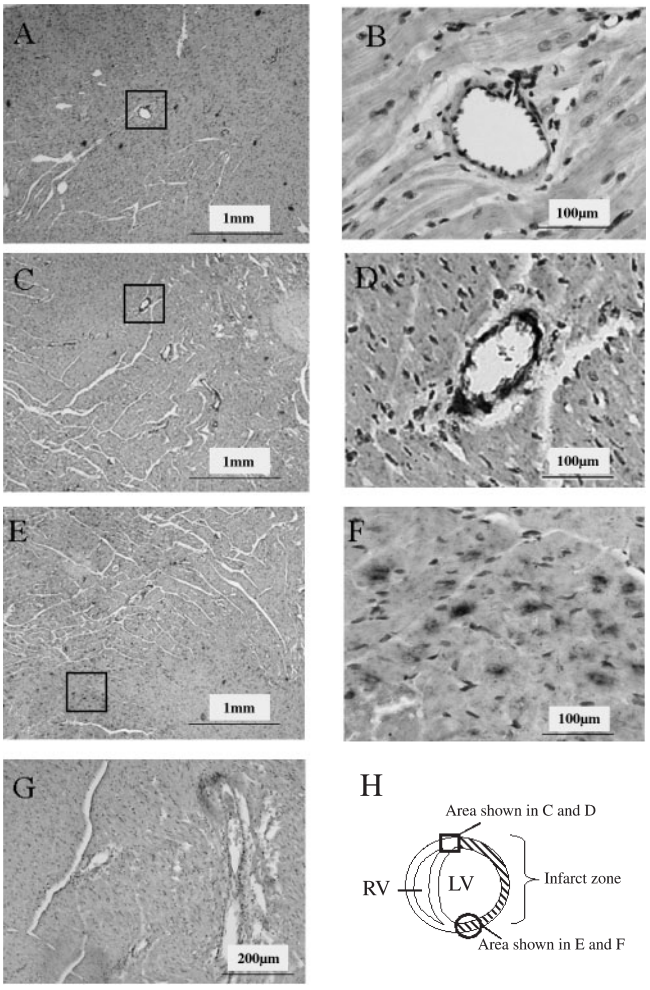

Fig. 3. ***In situ* hybridization analysis of ADAMTS1 mRNA in infarct endothelium and myocardium.** Anti-sense probe for ADAMTS1 mRNA was used for both sham-operated and infarct hearts. A: ADAMTS1 mRNA faintly detected in endothelium. B: Higher magnification of the square region in A. C: ADAMTS1 mRNA detected in the blood vessels in an infarct peripheral zone 3 h after MI. D: Higher magnification of the square region in C. E: ADAMTS1 mRNA detected in the myocardium in an infarct peripheral zone 3 h after MI. F: higher magnification of the square region in E. G: Sense probe for ADAMTS1 mRNA showed no signals in an infarct heart. H: schematic representation of the infarcted heart used for *in situ* hybridization analysis of ADAMTS1 mRNA. The hatched area represents the infarct zone. LV indicates left ventricle and RV indicates right ventricle. A scale bar is shown in each panel.

6 h, but reduced at 12 h and 24 h ($p < 0.05$). The relative level of VEGF mRNA showed similar kinetics to that of ADAMTS1 mRNA in the infarcted rat heart. ANOVA revealed that the alterations of both ADAMTS1 and type I collagen mRNAs after MI were significant.

In Situ Hybridization—Sham-operated hearts showed weak ADAMTS1 mRNA signals in endothelial cells (Fig. 3A). This observation matched those of previous reports (*34*). Using sense probe for ADAMTS1 mRNA, signals were not observed (Fig. 3G). Positive signals for ADAMTS1 mRNA were mainly observed around the infarct area, as schematically indicated in Fig. 3H.

Intense positive signals for ADAMTS1 mRNA were observed in some of the relatively large vascular endothelial cells in the infarct area at 3 h (Fig. 3C and D). These observations showed that not all endothelial cells expressed ADAMTS1 but more than half of those adjacent to the infarct zone expressed it. The endothelial cells in the right ventricle of 3-h MI rats expressed ADAMTS1 more strongly than those of sham-operated hearts (data not shown). Positive signals were also detected in the myocytes adjacent to the infarct zone (Fig. 3E and F). Neither myocytes in the central zone nor non-infarct myocytes in the right ventricle of infarcted hearts showed any positive signals for ADAMTS1. Normal myocytes in the sham-operated rats also showed no signals for ADAMTS1. Two series of experiments with different rats showed the identical signal distribution pattern. The results of *in situ* hybridization for ADAMTS1 mRNA in MI are summarized in Table 2. The endothelial cells adja-

Table 2. **The results of *in situ* hybridization for ADAMTS1 mRNA in MI.**

	Sham	MI-remote area	MI-infarct area
Endothelium	(±)	(+)	(+++)
Myocardium	(–)	(–)	(++)

The results of *in situ* hybridization were determined as described in "MATERIALS AND METHODS." Note that endothelium adjacent to the infarct zone strongly expressed ADAMTS1 mRNA. Normal myocytes and non-infarct myocytes did not show ADAMTS1 mRNA expressions while non-infarct endothelium expressed ADAMTS1 mRNA.

cent to the infarct zone showed the most intense signals for ADAMTS1 mRNA, and myocytes neighboring the infarct zone also showed ADAMTS1 mRNA signals. In contrast, in the non-infarct area (right ventricle of the infarcted heart), some endothelial cells showed positive signals for ADAMTS1 mRNA and no signals were observed in the non-infarct myocytes.

Western Blotting—Western blot analysis showed that ADAMTS-1 protein was actually produced in the infarcted heart (Fig. 4). The induction of ADAMTS-1 mRNA was found to be followed by the appearance of ADAMTS-1 protein after MI. At 6 h after coronary artery ligation, immunoreactive proteins with molecular masses of approximately 85 kDa and 110 kDa were detected. This result is in line with a previous report (*41*). A small unknown band was also seen, as previously reported (*41*). Without primary antibody, the samples showed only nonspecific bands that were similarly observed in all samples loaded (data not shown). Sham-operated heart did not show distinct bands (Fig. 4). The induction of ADAMTS-1 in the infarcted heart was transient, and ADAMTS-1 protein disappeared by 2 d after MI (Fig. 4). We also tested an anti-ADAMTS-1 polyclonal antibody against the synthetic peptide (^{333}CNWQKQHNPPSDRDAEYHD^{351}) which was previously used by another group (*41*). This antibody also reacted with the protein extracts from rat infarct heart (data not shown).

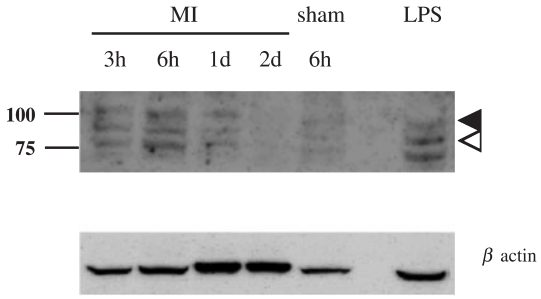

Fig. 4. **Western blot analysis was performed using A-19 antibody to examine the ADAMTS-1 protein in the infarct heart.** MI indicates infarct hearts, and sham indicates sham-operated heart. LPS indicates LPS-stimulated heart. An immuno-reactive 110 kDa protein (black arrowhead) was detected with maximal induction 6 h after ligation. A-19 antibody detected proteins of 110 kDa, 85 kDa (open arrowhead) and additional small size band. The positions of molecular size markers are indicated on the left side of the panel. The results for β-actin are shown at the bottom.

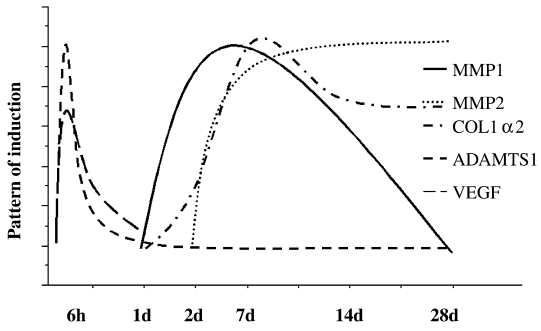

Fig. 5. **Time-dependent changes in the expression levels of ADAMTS1, VEGF, MMP-1, MMP-2 and α2(I) collagen in the infarcted heart.** Note that ADAMTS1 is clearly induced earlier than other MMPs and its induction pattern is similar to that of VEGF.

DISCUSSION

In this study, we found that ADAMTS1 mRNA was significantly up-regulated at 3 h after MI, and *in situ* hybridization revealed that distinct signals for ADAMTS1 mRNA in the infarct area.

ADAMTS1 Was Induced Immediately after Artery Ligation—Here we report the first observations about the level of ADAMTS metalloprotease in MI. Previous reports regarding MMPs in the infarcted heart demonstrated that MMPs such as MMP-1 and MMP-2 have roles in ventricular remodeling after MI. For example, Cleutjens *et al*. reported that MMP-1 mRNA reached its peak 7 d after MI, and that induction of MMP-1 was only observed in the infarcted myocardium (*12*). Our study demonstrated that type I collagen, the substrate for MMP-1, was increased in the infarcted area around 7 d (Fig. 2B). This result is in good agreement with the findings of previous studies (*12*). Deten et al. reported that other major MMPs, MMP-2 and MMP-9, were increased but that their mRNA levels were differently regulated after MI (*9*). In the infarcted heart, MMP-2 was initially diminished and then increased 6 d after infarction (*9*). In contrast, MMP-9 was increased at 6 h and reached its peak 1 d after MI. The change in the expression level of ADAMTS1 mRNA in the infarcted heart occurred more rapidly than the changes of expression of MMPs. The kinetics of ADAMTS1 in the infarcted heart seem to be different from those of MMPs (Fig. 5). We further confirmed that ADAMTS-1 protein was indeed expressed in the infarcted heart (Fig. 4). This is the first observation showing that the infarcted heart itself is a source of ADAMTS-1 protein.

ADAMTS1 Expression Shows a Pattern Distinct from Proteoglycan Expression in the Infarcted Heart—ADAMTS1 has been shown to be an active metalloprotease (*42*). Previous investigations showed that ADAMTS1 can digest proteoglycans (PGs) such as aggrecan and versican (*24*, *43*). However, aggrecan is a major PG in cartilage and was not highly expressed in either the normal or infarcted heart (data not shown). Sandy *et al*. reported that ADAMTS1 has the capacity to cleave the proteoglycan versican, which is one of the major PGs in the heart and vessels (*24*). We examined the pattern of expression of versican mRNA in rat MI heart and found that it was different from that of ADAMTS1 mRNA (Nakamura, K. and Hirohata, S., unpublished observations). Our previous

observations clearly showed that ADAMTS1 was induced and expressed differently from PGs in the infarcted heart (*6*, *44*). In fact, there are other ADAMTS metalloproteases, such as ADAMTS4 and ADAMTS5, that function in the cleavage of PGs. ADAMTS4 and ADAMTS5 mRNA levels also increased with a profile distinct from that of ADAMTS1 in the infarcted heart (Nakamura, K. and Hirohata, S., unpublished observations). The ECM degradation after MI is believed to occur in a later phase (*45*). Degradation of major ECM proteins such as collagen and PG after MI is initiated at least 12 h to 24 h after artery obstruction (*45*). At 3 h after coronary artery ligation, ischemic endothelium and myocardium may produce various cytokines and growth factors; however, the ventricular remodeling process does not take place at all. Moreover, myofibroblasts, which are the major source of ECM molecules, appear in a later phase, such as 7 d after infarction (*6*). These data indicate that the role of ADAMTS1, which was induced in the acute phase of MI and expressed by myocardium and endothelium, is unlikely to be versican degradation in the infarcted heart. Because ADAMTS1 is an active metalloprotease (*42*), it is also possible that some unknown substrate for ADAMTS1 is involved in the infarct area after infarction in rats.

ADAMTS1 Was Expressed after Myocardial Infarction—In this study, we identified the cells responsible for ADAMTS1 production in MI rats by *in situ* hybridization. There were two sources of ADAMTS1 production in the rat MI heart. Firstly, we found that ADAMTS1 mRNA accumulated in the endothelium adjacent to the infarct zone. Our finding that normal endothelial cells expressed ADAMTS1 is in line with previous studies. Diamantis *et al*. demonstrated by *in situ* RT-PCR analysis that ADAMTS1 mRNA was detected in normal liver endothelial cells and that ADAMTS1 mRNA was diminished in cirrhotic endothelial cells in rats (*34*). In contrast, ADAMTS1 mRNA was increased in a few hours in MI. Kuno *et al*. reported that ADAMTS1 was up-regulated by LPS stimulation (*23*). Bongrazio *et al*. reported that human umbilical vein endothelial cells expressed ADAMTS1 mRNA in response to laminar shear stress (*46*). The induction of ADAMTS1 was maximal 4 h after exposure to shear stress. These results together suggest that ADAMTS1 is induced in acute inflammation or stimulation (*e.g*., LPS stimulation, shear stress) but not in chronic inflammation (*e.g*., liver cirrhosis). Secondly, we found that myocytes immediately surrounding the infarct zone expressed ADAMTS1 mRNA, while normal myocytes did not. Neither non-infarct myocytes in the right ventricle nor myocytes in the infarct central zone showed any signals for ADAMTS1 mRNA. The fact that ADAMTS1 was exclusively expressed in the infarct zone led us to propose the hypothesis that surviving ischemic cells have a special mechanism (*e.g*., sensing and surviving ischemia) (*47*) and transient ADAMTS1 induction might be related to the rate of survival of myocytes after ischemia. Our observations showed for the first time that myocytes and endothelial cells can express ADAMTS proteases in cardiovascular disease.

ADAMTS1 and VEGF Exhibit Similar Temporal Patterns of mRNA Expression in the Infarcted Heart—Another potential role of ADAMTS1 is the regulation of angiogenesis via binding to VEGF (*27*). In this study, real-time RT-PCR demonstrated that VEGF was increased 6 h after artery obstruction (Fig. 2C). Our observation was consistent with a previous report (*22*) in which Li *et al*. found by Northern blot analysis that VEGF was increased in the infarcted myocardium at 6 h in rats (*22*). VEGF mRNA was strongly expressed by the myocytes immediately surrounding the infarct zone, while infarct myocytes did not express VEGF mRNA (*22*). As shown in Fig. 3F, myocardium neighboring the infarct zone strongly expressed ADAMTS1 mRNA. It was also reported that endothelial cells are another source of VEGF production after MI (*48*). Many reports have revealed that VEGF plays a role as an endogenous activator of coronary collateral formation in the human heart. Non-infarct ADAMTS1-positive endothelial cells may be related to the local VEGF action in the infarct remote area (*22*).

Conclusion—In conclusion, this is the first report showing strong induction of ADAMTS1 mRNA in a disease. The ischemic myocardium was shown to be a new source of ADAMTS1 mRNA, and endothelial cells, which weakly express ADAMTS1 mRNA in the normal heart, showed increased expression of this mRNA immediately after MI concomitant with VEGF expression.

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