*Original Article*

# The Significance of Chymase in the Progression of Abdominal Aortic Aneurysms in Dogs

Keiichi FURUBAYASHI[1),2)], Shinji TAKAI[1)], Denan JIN[1)], Michiko MURAMATSU[1)], Toshihiko IBARAKI[1)], Masayoshi NISHIMOTO[3)], Hitoshi FUKUMOTO[3)], Takahiro KATSUMATA[2)], and Mizuo MIYAZAKI[1)]

**In this study, we investigated the effect of a specific chymase inhibitor, NK3201, in the progression of abdominal aortic aneurysm in a dog experimental model. Abdominal aortic aneurysms were induced in dogs by injecting elastase into the abdominal aorta. NK3201 (1 mg/kg per day, p.o.) or a placebo was started 3 days before elastase injection and continued for 8 weeks after the injection. On abdominal ultrasound, the aortic diameter was seen to gradually expand in the placebo-treated group, but not in the NK3201-treated group. Eight weeks after elastase injection, the ratio of the medial area to the total area in the placebo-treated group was significantly smaller than that in the normal group, but it was significantly larger than that in the NK3201-treated group. In addition to chymase activity, angiotensin II–forming and matrix metalloproteinase-9 activities were significantly higher in the placebo-treated group than in the normal group; in the NK3201-treated group, all of these activities were significantly decreased. On immunohistochemical analyses, there was a significantly greater number of chymase-positive cells in the placebo-treated group than in the normal group, but the number was significantly smaller in the NK3201-treated group than in the placebo-treated group. Thus, chymase inhibition may become a useful strategy for preventing abdominal aortic aneurysms. (*Hypertens Res* 2007; 30: 349–357)**

***Key Words*: abdominal aortic aneurysm, angiotensin II, chymase, inhibitor, matrix metalloproteinase-9**

## Introduction

Abdominal aortic aneurysms (AAAs) are generally characterized by the widespread destruction of elastic lamellae in the media and by an inflammatory response in the vascular wall. The pathophysiology of AAAs includes aortic atherosclerosis, chronic inflammation within the outer aortic wall, and an imbalance between the production and degradation of structural extracellular matrix proteins (*1*–*3*). Ruptured AAAs have a high mortality rate unless the appropriate diagnosis is made, since only timely surgery can benefit patients with AAAs. However, aged patients with AAAs who have a poor quality of life due to a mental or physical handicap are not surgical candidates. Therefore, there is a need for effective pharmacotherapy for AAAs.

The destruction of elastin is considered to be one of the major pathogenetic mechanisms of AAAs. Although elastic fibers normally maintain the structure of the vascular wall against hemodynamic stress, enzymatic degradation may induce remodeling of the extracellular matrix, resulting in aneurysmal development and finally rupture. Chymase is a chymotrypsin-like serine protease located in the secretory granules of mast cells. Dog chymase converts promatrix met-

From the [1)]Department of Pharmacology and [2)]Department of Thoracic and Cardiovascular Surgery, Osaka Medical College, Takatsuki, Japan; and [3)]Osaka Mishima Critical Care Medical Center, Takatsuki, Japan.

This study was partly supported by the Novartis Foundation (Japan) and the Japanese Research Foundation for Clinical Pharmacology. This study was partly supported by a grant from the Novartis Foundation (Japan) to the Japanese Research Foundation for Clinical Pharmacology.

Address for Reprints: Shinji Takai, Ph.D., Department of Pharmacology, Osaka Medical College, 2–7 Daigaku-machi, Takatsuki 569–8686, Japan. E-mail: pha010@art.osaka-med.ac.jp

Received August 25, 2006; Accepted in revised form December 4, 2006.

alloproteinase (proMMP)-9 to matrix metalloproteinase (MMP)-9 (*4*). Previous studies in mice have demonstrated that the various expressions of MMP-9 are associated with the development of AAAs through elastic fiber disruption (*5*, *6*). For example, an MMP inhibitor limits the expansion of experimental AAAs, and targeted gene disruption of MMP-9 suppresses AAA development (*5*, *6*). Thus, increased activity of MMP-9 may play an important role in the development of AAAs.

Chymase converts angiotensin I to angiotensin II in cardiovascular tissues (*7*–*9*). Previously, we have demonstrated that chymase activity was significantly higher in AAAs than in normal aortas (*10*, *11*). Daugherty *et al*. (*12*) suggested that angiotensin II is related to aortic aneurysms, because angiotensin II promotes aortic aneurysms in apolipoprotein E–deficient mice. In fact, angiotensin II blockade might have inhibitory effects on the aneurysmal formation (*13*). On the other hand, we demonstrated that a chymase inhibitor also prevents the development of AAA in hamsters (*14*). However, it has been unclear whether chymase is involved in MMP-9 activation in AAAs. In this study, we wanted to confirm whether chymase inhibition is involved in the suppression of MMP-9 in a dog AAA model.

## Methods

### Drugs

2-(5-Formylamino-6-oxo-2-phenyl-1,6-dihydropyrimidine-1-yl)-*N*-[{3,4-dioxo-1-phenyl-7-(2-pyridyloxy)}-2-heptyl]acetamide (NK3201) was synthesized as a specific chymase inhibitor (Nippon Kayaku Co. Ltd., Tokyo, Japan) (*15*).

### Experimental Model

Fifteen male beagle dogs weighing 9–11 kg were obtained from Japan SLC (Shizuoka, Japan) and were randomly divided into 3 groups before the operation. To create the AAA model, under pentobarbital anesthesia (35 mg/kg i.v.), the abdominal aorta from the infrarenal aorta to the bifurcation of the aorta was isolated using a retroperitoneal approach. Temporary forcep blocks were placed at the proximal and distal portions of the aorta (the distance between the 2 portions was 5 cm). From the lumbar artery, 400 units/ml of porcine pancreatic elastase (type I; SERVA Electrophoresis GmbH, Heidelberg, Germany) in saline were injected into the aorta and incubated for 2 h. Beginning 3 days before the elastase was given and continuing until the end of the experiment, each dog was given the chymase inhibitor NK3201 (1 mg/kg once daily, p.o., $n=5$) or placebo ($n=5$). In the placebo-treated group, the placebo consisted of the same volume of 0.5% carboxymethylcellulose as was given to the treated dogs. Untreated dogs constituted the normal group ($n=5$). We measured the external diameter of the AAAs using an abdominal ultrasound pre-operatively, postoperatively, and every 2 weeks after elastase injection for a total of 8 weeks. Eight weeks after elastase injection, the aortas were removed. The experimental procedures for the animals were conducted in accordance with the guidelines of Osaka Medical College. All analyses were conducted in a blinded manner.

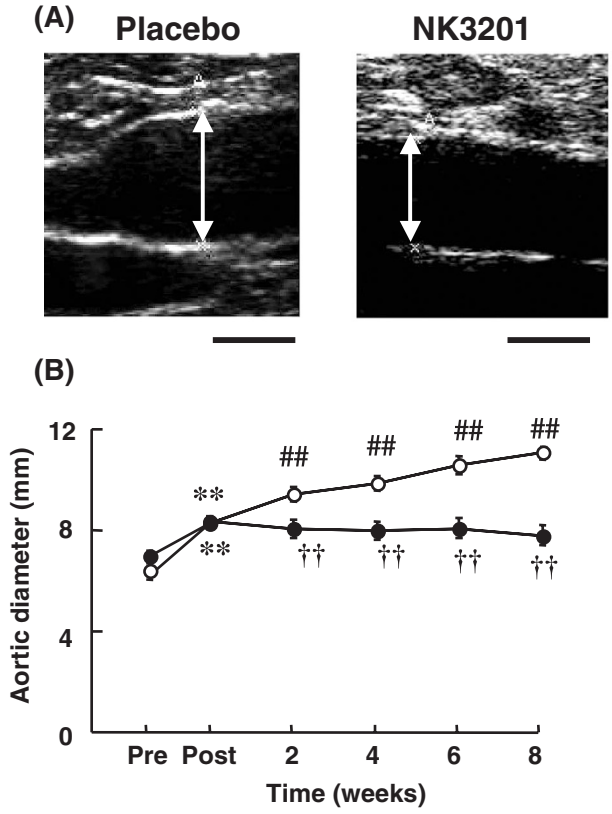


**Fig. 1.** *A: Photographs of a typical ultrasound image of a placebo-treated dog and an NK3201-treated dog 8 weeks after elastase injection. B: Aortic diameters in the placebo (open circle)- and NK3201 (closed circle)-treated groups during the experiment. "Pre" and "Post" indicate data obtained before and just after elastase injection, respectively.* **$p<0.01$ vs. *"Pre" in each group.* ##$p<0.01$ vs. *"Post" in each group.* ††$p<0.01$ vs. *the placebo-treated group at the same time point after the elastase injection. Scale bars represent 5 mm.*

### Aortic Diameter by Ultrasound Imaging

The aortic diameter was assessed at the level of the abdominal aorta by ultrasound imaging (*16*). Measurements were made using a 2-dimensional image perpendicular to the long axis of the aorta in views showing the largest aortic diameter. The maximal luminal width was measured at each aortic level.

### Histological Analysis

The aortic segments were fixed in 10% methanol Carnoy's

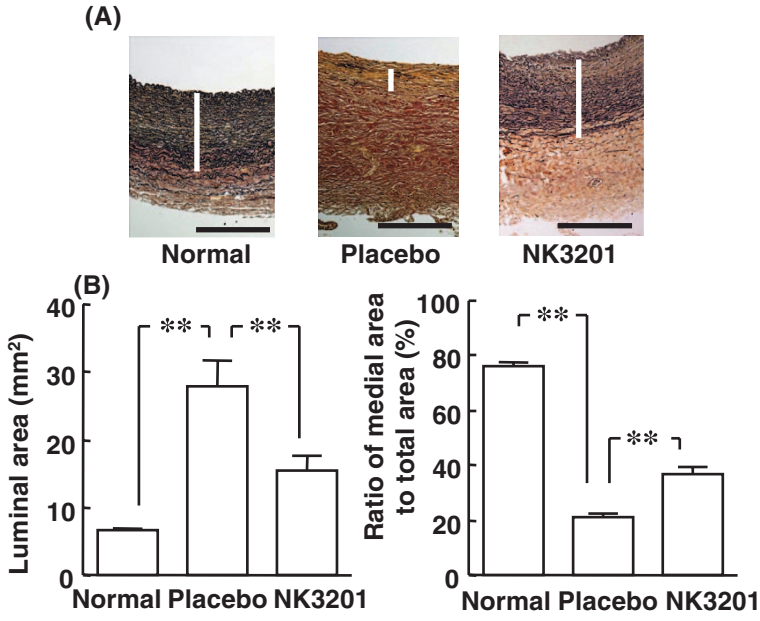


**Fig. 2.** *A: Typical photographs of van Gieson's elastin-stained slices of aortas obtained from the normal, placebo- and NK3201-treated dogs 8 weeks after elastase injection. White bars indicate the medial layer. B: Luminal areas and ratios of the medial area to total area (%) in the normal, placebo-, and NK3201-treated groups 8 weeks after elastase injection.* $^{**}p < 0.01$ vs. *the placebo-treated group. Scale bars represent 200 μm.*

fixative overnight and embedded in paraffin. Then, 3-μm-thick sections were cut from each block. The sections were stained with both hematoxylin-eosin and van Gieson's elastin stain. The luminal area and the ratio of the medial area to the total area of the aorta were measured using a computerized morphometry system, MacSCOPE Ver. 2.2 (Mitani Co., Fukui, Japan).

To determine the degree of chymase-positive cell accumulation in the AAAs, we performed immunohistochemical staining on 5 sections using anti–dog chymase antibody (a gift from Caughey), as previously described (*17*, *18*). To elucidate the inflammatory process, neutrophils were immunostained with anti-neutrophil antibody (PMN 8C7; ICN Biomedicals. Inc., Costa Mesa, USA) (*19*). The numbers of chymase-positive cells and neutrophils were determined, using the computerized morphometry system, and were expressed as the number of stained cells per $mm^2$.

## Preparation of Vascular Tissue

The abdominal aorta was homogenized in 20 mmol/l Na-phosphate buffer, pH 7.4. The homogenate was centrifuged at 20,000 rpm for 30 min. The supernatant was discarded, and the pellets were re-suspended and homogenized in 10 mmol/l Na-phosphate buffer, pH 7.4, containing 0.5 mol/l NaCl and 1% Triton X-100. Next, the homogenate was centrifuged at 20,000 rpm for 30 min, and the supernatant was used for measuring angiotensin II–forming, chymase and angiotensin-converting enzyme (ACE) activities (*20*). The remaining aorta was homogenized in 50 mmol/l Tris-HCl buffer, pH 7.5, containing 1.0 mol/l NaCl, 2.0 mol/l urea, 0.1% Brij-35, 0.1% ethylenediaminetetraacetic acid (EDTA), and a mixture of serine, cysteine, and aspartic protease inhibitors (protease inhibitor cocktail; Sigma Chemical Co., St. Louis, USA). The homogenate was centrifuged at 20,000 rpm for 30 min, and the supernatant was used for the measurement of MMP activity.

## Angiotensin II–Forming, Chymase and ACE Activities

Aortic extracts, prepared as previously described, were used to measure chymase and angiotensin II–forming activities (*21*). Chymase activity was measured by incubating the tissue extracts for 1 h at 37°C with 4 mmol/l angiotensin I in 150 mmol/l borax-borate buffer, pH 8.5, containing 5 mmol/l EDTA, 8 mmol/l dipyridyl, and 0.77 mmol/l diisopropylfluorophosphate (*21*). ACE activity was measured by incubating the tissue extracts for 1 h at 37°C with 5 mmol/l hippuryl-His-Leu in 250 μl of 10 mmol/l phosphate buffer, pH 8.3, containing 300 mmol/l NaCl (*22*). Angiotensin II–forming activity was measured by incubating the tissue extracts for 1 h at 37°C with 4 mmol/l angiotensin I (*21*).

Protein concentration was assayed with BCA Protein Assay Reagents (Pierce, Rockford, USA), using bovine serum albumin as a standard.

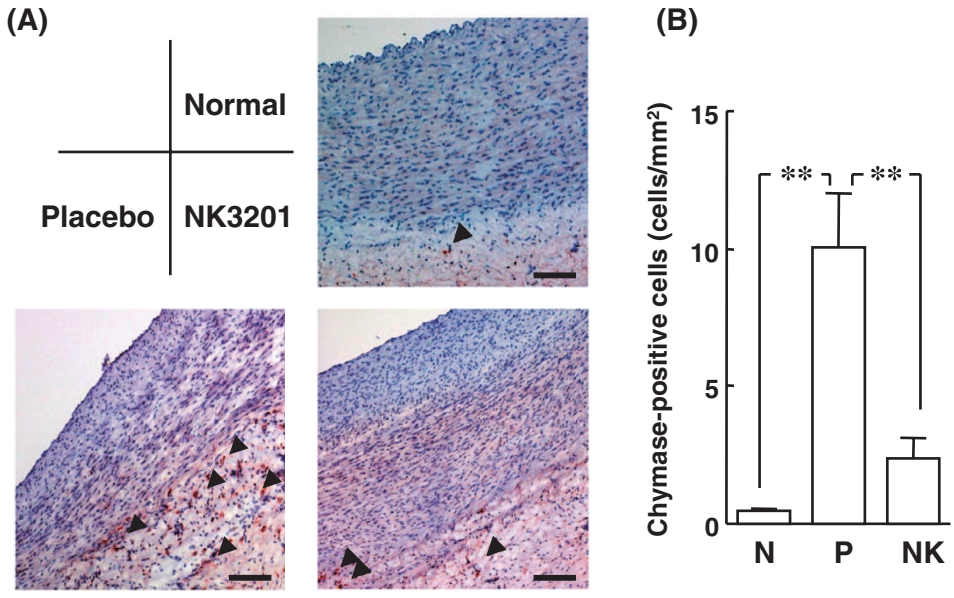


**Fig. 3.** *A: Typical photographs of aorta slices stained with anti-chymase antibody obtained from the normal, placebo- and NK3201-treated groups 8 weeks after elastase injection. Arrowheads indicate chymase-positive cells. B: The numbers of chymase-positive cells in the normal (N), placebo (P)- and NK3201 (NK)-treated groups 8 weeks after elastase injection.* $^{**}p < 0.01$ *vs. the placebo-treated group. Scale bars represent 100 μm.*

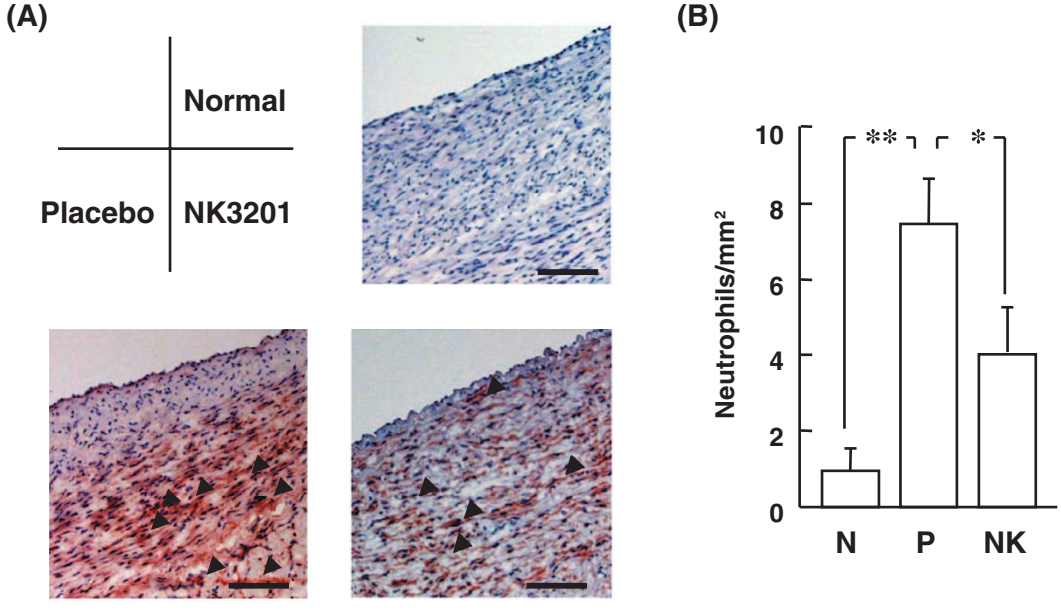


**Fig. 4.** *A: Typical photographs of aorta slices stained with anti-neutrophil antibody obtained from the normal, placebo- and NK3201-treated groups 8 weeks after elastase injection. Neutrophils are indicated by arrowheads. B: The numbers of neutrophils in the normal (N), placebo (P)- and NK3201 (NK)-treated groups 8 weeks after elastase injection.* $^{**}p < 0.01$ *vs. the placebo-treated group. Scale bars represent 100 μm.*

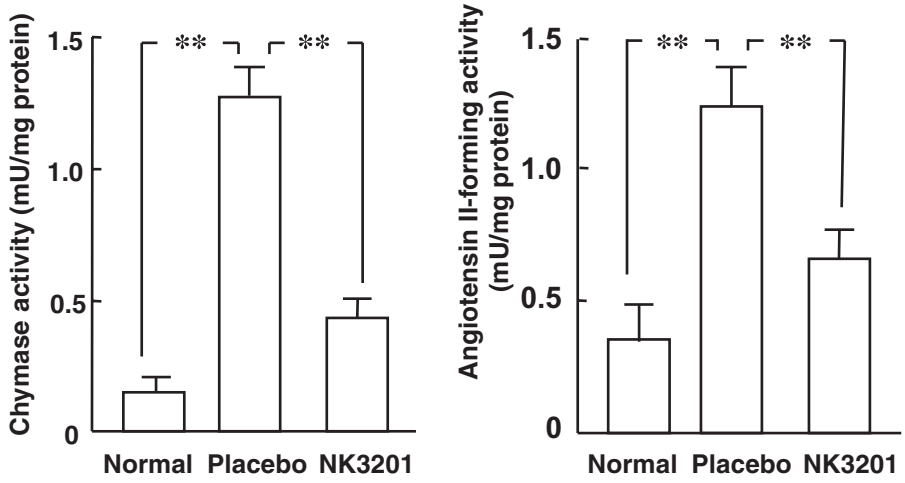


**Fig. 5.** *Chymase and angiotensin II–forming activities of the aorta obtained from the normal, placebo-, and NK3201-treated groups 8 weeks after elastase injection.* **p < *0.01* vs. *the placebo-treated group.*

### MMP-2 and MMP-9 Activities

In the dog AAA model, MMP-2 and MMP-9 activities in the dog AAA model were assessed as described previously (*4*). Equal volumes of the tissue extract (200 μg) were diluted with sodium dodecyl sulfate (SDS). MMP-2 and MMP-9 activities were detected using standard gelatin zymography with SDS-polyacrylamide gel electrophoresis containing 1 mg/ml gelatin.

### Statlstical Analysis

Significant differences between the mean values of the 2 groups were evaluated by Student's *t*-test for unpaired data. Significant differences among the mean values of multiple groups were evaluated using 1-way ANOVA followed by Fisher's test. Values were considered statistically significant at $p<0.05$. Data are expressed as the mean±standard error of the mean.

## Results

### Effects of NK3201 on Aortic Diameter

Figure 1A shows typical ultrasound images of a placebo-treated dog and an NK3201-treated dog 8 weeks after elastase injection. The aortic diameters of the placebo-treated and NK3201-treated groups were not significantly different before elastase injection (Fig. 1B). Just after elastase injection, the aortic diameters in both the placebo- and NK3201-treated groups enlarged to the same extent; at that time, there was no significant difference in aortic diameters between these 2 groups. However, 2 weeks after elastase injection, the aortic diameter was significantly larger in the placebo-treated group than it had been just after the elastase injection; the increase in the diameter progressed in a time-dependent manner. On the other hand, in the NK3201-treated group, compared to the aortic diameter just after elastase injection, the aortic diameter did not increase even 8 weeks after elastase injection.

### Effects of NK3201 on Luminal Area and Medial Area

Typical photographs of aortas isolated from the normal, placebo-, and NK3201-treated groups 8 weeks after elastase injection are shown in Fig. 2A. The luminal area was significantly larger in the placebo-treated group than in the normal group. However, the luminal area was significantly smaller in the NK3201-treated group than in the placebo-treated group (Fig. 2B). On the other hand, the ratio of the medial area to the total area in the placebo-treated group was significantly smaller than that in the normal group. However, the ratio of the medial area to the total area in the NK3201-treated group was larger than that in the placebo-treated group (Fig. 2B).

### Effects of NK3201 on Chymase-Positive Cells and Neutrophils

On immunohistochemistry, there were many chymase-positive cells in the adventitia in the placebo-treated group 8 weeks after elastase injection, though there were few such cells in the other groups (Fig. 3A). In the placebo-treated group, the number of chymase-positive cells per $mm^2$ was significantly higher than that in the normal group (normal group, 0.49±0.1; placebo-treated group, 10.0±2.0; Fig. 3B). On the other hand, the number of chymase-positive cells per $mm^2$ (2.39±0.7) was significantly lower in the NK3201-treated group than in the placebo-treated group.

Many neutrophils were also detected in the adventitia in the

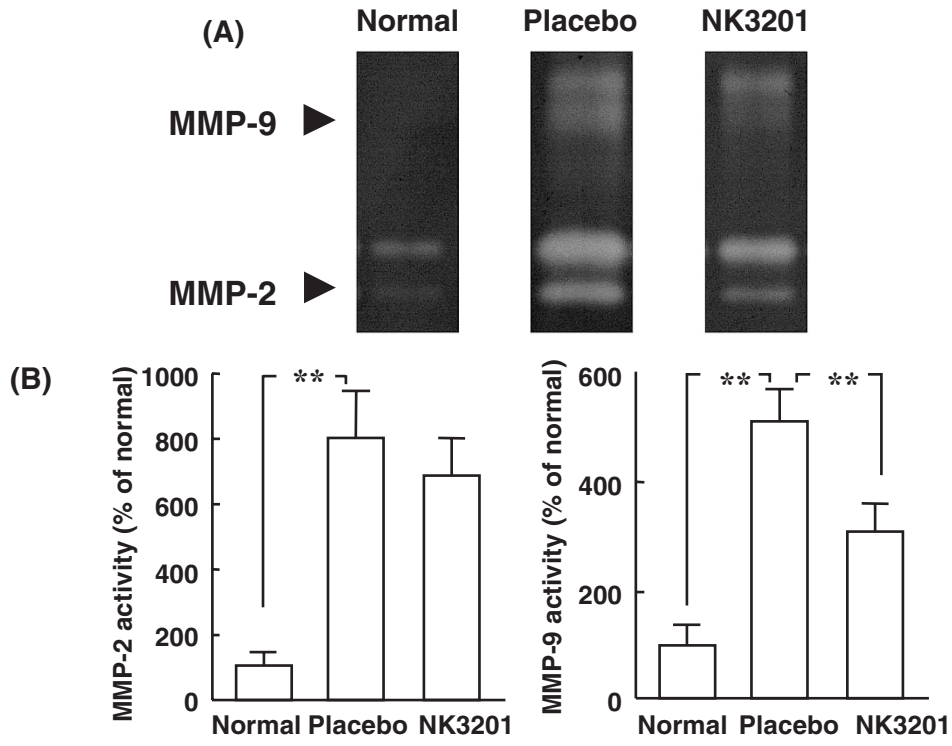


**Fig. 6.** *A: Typical photographs on gelatin zymography. B: MMP-2 and MMP-9 activities in the normal, placebo-, and NK3201-treated groups 8 weeks after elastase injection.* **p < *0.01* vs. *the placebo-treated group.*

placebo-treated group, but there were few such cells in the other groups (Fig. 4A). The neutrophil number per $mm^2$ was significantly higher in the placebo-treated group than in the normal group (normal group, 0.96±0.59; placebo-treated group, 7.49±1.19; Fig. 4B). However, in the NK3201-treated group, the neutrophil number per $mm^2$ (4.04±1.23) was significantly lower than in the placebo-treated group.

### Effects of NK3201 on Aortic Enzyme Activity

Chymase activity was significantly increased in the placebo-treated group compared to the normal group 8 weeks after elastase injection (Fig. 5). On the other hand, the chymase activity in the NK3201-treated group was significantly lower than that in the placebo-treated group. ACE activity was not significantly different among the 3 groups (data not shown).

Eight weeks after elastase injection, total angiotensin II–forming activity was significantly higher in the placebo-treated group than in the normal group. However, total angiotensin II–forming activity was significantly decreased in the NK3201-treated group compared to the placebo-treated group (Fig. 5).

Both MMP-2 and MMP-9 activities were significantly increased in the placebo-treated group as compared to the normal group. MMP-9 activity was significantly lower in the NK3201-treated group than in the placebo-treated group (Fig. 6). MMP-2 activity tended to be lower in the NK3201-treated group than in the placebo-treated group, though the difference did not reach the level of statistical significance.

## Discussion

We evaluated the preventive effect of a chymase inhibitor on the progression of canine AAAs. The chymase inhibitor NK3201 has been shown to be an orally active and specific chymase inhibitor, inhibiting human, dog and hamster chymases by $IC_{50}$ at concentrations of 2.5, 1.2 and 28 nmol/l, respectively (*15*). On the other hand, NK3201 has no inhibitory activity against other types of serine proteases—*i.e.*, tryptase, thrombin, elastase, plasmin or plasminogen activator (*15*). In the present study, MMP-2 and MMP-9 activities in the extract were not inhibited directly by concentrations of NK3201 up to 100 μmol/l (data not shown). In the above-described study, the concentrations of NK3201 in the plasma, heart and aorta of dogs were about 470, 195 and 78 nmol/l at 8 h after oral administration of the drug (1 mg/kg), respectively, and NK3201 was also detected at a concentration of over 10 nmol/l in the heart, aorta, lung, liver, kidney, muscle, and skin, but not in the brain, at 24 h after oral administration (*15*). These findings suggest that oral administration of over 1 mg/kg of NK3201 inhibits chymase activity in tissues *in vivo*. In the present study, just after injecting elastase, a significant enlargement of aortic diameter was seen in both the placebo- and the NK3201-treated groups; however, there was no sig-

nificant difference between the groups in the degree of enlargement. This result shows that NK3201, a chymase inhibitor, could not inhibit the activity of the injected elastase. On the other hand, after the injection, the aortic diameter gradually increased in the placebo-treated group but not in the NK3201-treated group. Therefore, NK3201 could not inhibit the elastase-induced expansion of the aorta, but it could prevent the progression of AAAs after elastase injection. At the end of the experiment, chymase activity was found to be significantly higher in the placebo-treated group than in the normal group; in addition, angiotensin II–forming and MMP-9 activities were significantly increased in the placebo-treated group compared to the normal group. However, in the chymase inhibitor–treated group, these activities were significantly decreased compared to those in the placebo-treated group. These findings suggest that chymase plays an important role in the progression of AAAs by increasing not only angiotensin II but also MMP-9.

Extracellular matrix–degrading peptidases are found in AAAs. In particular, MMP-9 is elevated in human AAAs (*1*–*3*). Previously, dog and mouse chymases have been reported to directly activate proMMP-9 to MMP-9 *in vitro* (*4*, *23*). In the present study, not only MMP-9 activity but also MMP-2 activity in the placebo-treated dogs was significantly increased compared to the activity level seen in normal dogs. MMP-9 activity was significantly decreased by treatment with NK3201. MMP-2 activity also tended to be decreased by NK3201 treatment, though the decrease was not statistically significant. MMP-2 and MMP-9 weaken the extracellular matrix and appear to be pivotal for the migration of vascular smooth muscle cells (VSMCs); in addition, they may contribute to AAA formation (*24*, *25*). In the present study, the aortic media was clearly maintained by treatment with NK3201, as shown in Fig. 2A. Therefore, prevention of VSMC migration may be part of the mechanism by which NK3201 prevents AAAs.

In human AAAs, we have previously shown that there is an increase of chymase activity in the adventitia of vessels (*10*, *11*). In our dog AAA model in the present study, many chymase-positive cells were detected in the adventitia of the aorta. In the dog AAAs, mast cells stained by toluidine blue were located in areas that were almost completely consistent with the expression of chymase-positive cells (data not shown); this indicates that mast cells are the main source of chymase, as has been previously reported (*18*). The number of chymase-positive cells in the aortas of the placebo-treated dogs was significantly higher than that in the normal dogs, while the number of chymase-positive cells was significantly decreased in the aortas of NK3201-treated dogs. It is also known that chymase is stored in mast cells. On the other hand, chymase plays an important role in the accumulation of mast cells by activating stem cell factor (*26*). The dose of NK3201 (1 mg/kg per day) used in the present study was also used in our previous study, where we found that NK3201 significantly reduced the number of chymase-positive cells (*18*). In cardiomyopathic hamsters, the number of mast cells in the heart was also decreased by treatment with a chymase inhibitor, BCEAB (*27*). Therefore, the decrease in chymase activity due to NK3201 might be involved in the decrease of the number of mast cells that contain chymase.

Angiotensin II induces an inflammatory cytokine, interleukin-1, and a chemokine, monocyte chemoattractant protein (MCP)-1 (*28*, *29*). Subsequently, interleukin-1, which is produced by activated macrophages, induces tissue damage. MCP-1 induces the activation and migration of monocytes, resulting in macrophage accumulation (*30*, *31*). An angiotensin II type 1 receptor blocker (ARB) was found to reduce gene expression of MCP-1 and to reduce the accumulation of macrophages (*32*). Daugherty *et al*. (*12*) demonstrated that infusion of angiotensin II led to the development of aortic aneurysms in apolipoprotein E–deficient mice. Ejiri *et al*. (*13*) reported that an ARB might have inhibitory effects on the aneurysmal formation, and angiotensin II induced by chymase may play an important role in the development of aortic aneurysms.

In human AAAs, macrophages are the predominant inflammatory cell types, though neutrophils are also detected (*33*). Elastic fibers provide structural integrity to the aortic wall. When elastic fibers are degraded, the elastic fragments become potent chemoattractants for monocytes and neutrophils (*33*, *34*). The role that neutrophils play in the initial stages of human AAAs is unclear. However, in the rat elastase-induced AAA model, neutrophils may play a very important role during the early inflammatory response and during aneurysm enlargement (*35*). In our dog AAA model, a high density of neutrophils was observed in the aorta of the placebo-treated group even 8 weeks after elastase injection. In the rat model, 12 days after elastase injection, the number of inflammatory cells present in the aorta decreased, and further enlargement of the aneurysm was not observed (*36*). On the other hand, in our dog AAA model, aneurysmal enlargement appeared to continue even 8 weeks after elastase injection. In this study, we started giving NK3201 to the dogs 3 days before elastase injection; however, in clinical practice, the drugs would have to be given to patients who already have AAAs. Our model may be useful to study the effects of drugs on already formed AAAs; further research into the effects of NK3201 against already formed AAAs is needed.

In conclusion, chymase may be an important contributor to the increase of MMP-9 activity. Chymase is involved in the formation of angiotensin II and MMP-9, both of which strongly promote the progression of AAAs. Therefore, chymase inhibition may become a useful strategy for preventing AAAs.

## References

1. Thompson RW, Holmes DR, Mertens RA, *et al*: Production and localization of 92-kilodalton gelatinase in abdominal aortic aneurysms. An elastolytic metalloproteinase

expressed by aneurysm-infiltrating macrophages. *J Clin Invest* 1995; **96**: 318–326.
2. Freestone T, Turner RJ, Coady A, Higman DJ, Greenhalgh RM, Powell JT: Inflammation and matrix metalloproteinases in the enlarging abdominal aortic aneurysm. *Arterioscler Thromb Vasc Biol* 1995; **15**: 1145–1151.
3. McMillan WD, Tamarina NA, Cipollone M, Johnson DA, Parker MA, Pearce WH: Size matters: the relationship between MMP-9 expression and aortic diameter. *Circulation* 1997; **96**: 2228–2232.
4. Fang KC, Raymond WW, Blount JL, Caughey GH: Dog mast cell α-chymase activates progelatinase B by cleaving the Phe88-Gln89 and Phe91-Glu92 bonds of the catalytic domain. *J Biol Chem* 1997; **272**: 25628–25635.
5. Pyo R, Lee JK, Shipley JM, *et al*: Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. *J Clin Invest* 2000; **105**: 1641–1649.
6. Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter BT: Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. *J Clin Invest* 2002; **110**: 625–632.
7. Urata H, Kinoshita A, Misono K, Bumpus FM, Husain A: Identification of a highly specific chymase as the major angiotensin II–forming enzyme in the human heart. *J Biol Chem* 1990; **265**: 22348–22357.
8. Takai S, Shiota N, Sakaguchi M, Muraguchi H, Matsumura E, Miyazaki M: Characterization of chymase from human vascular tissues. *Clin Chim Acta* 1997; **265**: 13–20.
9. Caughey GH, Raymond WW, Wolters PJ: Angiotensin II generation by mast cell α- and β-chymases. *Biochim Biophys Acta* 2000; **1480**: 245–257.
10. Nishimoto M, Takai S, Fukumoto H, *et al*: Increased local angiotensin II formation in aneurysmal aorta. *Life Sci* 2002; **71**: 2195–2205.
11. Tsunemi K, Takai S, Nishimoto M, *et al*: Possible roles of angiotensin II–forming enzymes, angiotensin converting enzyme and chymase-like enzyme, in the human aneurysmal aorta. *Hypertens Res* 2002; **25**: 817–822.
12. Daugherty A, Manning MW, Cassis LA: Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E–deficient mice. *J Clin Invest* 2000; **105**: 1605–1612.
13. Ejiri J, Inoue N, Tsukube T, *et al*: Oxidative stress in the pathogenesis of thoracic aortic aneurysm: protective role of statin and angiotensin II type 1 receptor blocker. *Cardiovasc Res* 2003; **59**: 988–996.
14. Tsunemi K, Takai S, Nishimoto M, *et al*: A specific chymase inhibitor, 2-(5-formylamino-6-oxo-2-phenyl-1,6-dihydropyrimidine-1-yl)-*N*-[[3,4-dioxo-1-phenyl-7-(2-pyridyloxy)]-2-heptyl]acetamide (NK3201), suppresses development of abdominal aortic aneurysm in hamsters. *J Pharmacol Exp Ther* 2004; **309**: 879–883.
15. Takai S, Miyazaki M: Application of a chymase inhibitor, NK3201, for prevention of vascular proliferation. *Cardiovasc Drug Rev* 2003; **21**: 185–198.
16. Cecconi M, Manfrin M, Moraca A, *et al*: Aortic dimensions in patients with bicuspid aortic valve without significant valve dysfunction. *Am J Cardiol* 2005; **95**: 292–294.
17. Nishimoto M, Takai S, Kim S, *et al*: Significance of chymase-dependent angiotensin II–forming pathway in the development of vascular proliferation. *Circulation* 2001; **104**: 1274–1279.
18. Jin D, Ueda H, Takai S, *et al*: Effect of chymase inhibition on the arteriovenous fistula stenosis in dogs. *J Am Soc Nephrol* 2005; **16**: 1024–1034.
19. Todd MB, Waldron JA, Jennings TA, *et al*: Loss of myeloid differentiation antigens precedes blastic transformation in chronic myelogenous leukemia. *Blood* 1987; **70**: 122–131.
20. Kirimura K, Takai S, Jin D, *et al*: Role of chymase-dependent angiotensin II formation in regulating blood pressure in spontaneously hypertensive rats. *Hypertens Res* 2005; **28**: 457–464.
21. Jin D, Takai S, Sakaguchi M, Okamoto Y, Muramatsu M, Miyazaki M: An antiarrhythmic effect of a chymase inhibitor after myocardial infarction. *J Pharmacol Exp Ther* 2004; **309**: 490–497.
22. Kanemitsu H, Takai S, Tsuneyoshi H, *et al*: Chymase inhibition prevents cardiac fibrosis and dysfunction after myocardial infarction in rats. *Hypertens Res* 2006; **29**: 57–64.
23. Tchougounova E, Lundequist A, Fajardo I, Winberg JO, Abrink M, Pejler G: A key role for mast cell chymase in the activation of pro-matrix metalloprotease-9 and pro-matrix metalloprotease-2. *J Biol Chem* 2005; **280**: 9291–9296.
24. Vine N, Powell JT: Metalloproteinases in degenerative aortic disease. *Clin Sci* 1991; **81**: 233–239.
25. Knox JB, Sukhova GK, Whittemore AD, Libby P: Evidence for altered balance between matrix metalloproteinases and their inhibitors in human aortic diseases. *Circulation* 1997; **95**: 205–212.
26. Longley BJ, Tyrrell L, Ma Y, *et al*: Chymase cleavage of stem cell factor yields a bioactive, soluble product. *Proc Natl Acad Sci U S A* 1997; **94**: 9017–9021.
27. Takai S, Jin D, Sakaguchi M, *et al*: A novel chymase inhibitor, 4-[1-([bis-(4-methyl-phenyl)-methyl]-carbamoyl)3-(2-ethoxy-benzyl)-4-oxo-azetidine-2-yloxy]-benzoic acid (BCEAB), suppressed cardiac fibrosis in cardiomyopathic hamsters. *J Pharmacol Exp Ther* 2003; **305**: 17–23.
28. Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T: Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine-inducible enhancers. *FASEB J* 1995; **9**: 899–909.
29. Hernandez-Presa M, Bustos C, Ortego M, *et al*: Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-κB activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. *Circulation* 1997; **95**: 1532–1541.
30. Chen XL, Tummala PE, Olbrych MT, Alexander RW, Medford RM: Angiotensin II induces monocyte chemoattractant protein-1 gene expression in rat vascular smooth muscle cells. *Circ Res* 1998; **83**: 952–959.
31. Mabuchi T, Kitagawa K, Ohtsuki T, *et al*: Contribution of microglia/macrophages to expansion of infarction and response of oligodendrocytes after focal cerebral ischemia in rats. *Stroke* 2000; **31**: 1735–1743.
32. Hilgers KF, Hartner A, Porst M, *et al*: Monocyte chemoattractant protein-1 and macrophage infiltration in hypertensive kidney injury. *Kidney Int* 2000; **58**: 2408–2419.
33. Fontaine V, Jacob MP, Houard X, *et al*: Involvement of the

mural thrombus as a site of protease release and activation in human aortic aneurysms. *Am J Pathol* 2002; **161**: 1701–1710.
34. Senior RM, Griffin GL, Mecham RP: Chemotactic activity of elastin-derived peptides. *J Clin Invest* 1980; **66**: 859–862.
35. Hance KA, Tataria M, Ziporin SJ, Lee JK, Thompson RW: Monocyte chemotactic activity in human abdominal aortic aneurysms: role of elastin degradation peptides and the 67-kD cell surface elastin receptor. *J Vasc Surg* 2002; **35**: 254–261.
36. Anidjar S, Dobrin PB, Eichorst M, Graham GP, Chejfec G: Correlation of inflammatory infiltrate with the enlargement of experimental aortic aneurysms. *J Vasc Surg* 1992; **16**: 139–147.