A novel rat model of abdominal aortic aneurysm using a combination of intraluminal elastase infusion and extraluminal calcium chloride exposure

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Objective: **An ideal animal model of abdominal aortic aneurysm (AAA) is of great importance for clarifying unknown complex mechanisms of the pathogenesis. We introduce a new, simple technique to create reliable AAAs that simulate human aneurysms.**

Methods: **Experimental models of AAAs were created in 71 rats by means of a 20-minute application of intraluminal elastase (30 U) and extraluminal calcium chloride (0.5M) in the 1-cm segment of infrarenal abdominal aorta (group EC, n = 26). A single application of elastase (group E, n = 24) or calcium chloride (group C, n = 21) was used as control. The treated aorta in each group was measured under physiologic conditions and harvested at 1 and 4 weeks. Successful AAA formation was defined as a dilation ratio >50%. Inflammatory response, elastolytic activity, and histology in the treated aorta were evaluated among the three groups.**

Results: **The surgical procedure in each group was similarly completed for approximate 30 minutes and performed without any technical failure or operative death. At 4 weeks, the dilation ratio and wall thickness were 94.8% ± 9.9% and 125.4 ± 5.6 μm in group EC, 43.3% ± 6.3% and 149.6 ± 6.5 μm in group E, and 10.9% ± 4.2% and 152.9 ± 7.2 μm in group C. The success rate of AAA formation in group EC (92.7%) was significantly higher than that in group E (25.0%) and group C (0.0%). Less elastin content in the aortic wall was observed in group EC. At 1 week, tumor necrosis factor-α and interleukin-1β messenger RNA (mRNA) expressions were significantly upregulated, and CD3+ and CD11b+ cells were significantly infiltrated into the treated aorta of group EC, compared with groups E or C. Gelatinolytic activities and mRNA expressions of matrix metalloproteinase (MMP)-2 and MMP-9 were also significantly activated in group EC.**

Conclusion: **The rat AAA model using a combination of intraluminal elastase infusion and extraluminal calcium chloride exposure is simple and easy to perform and is highly reliable and reproducible to create a saccular aneurysm similar to human AAAs. This model could be more useful to clarify AAA pathogenesis, mechanisms, and treatment interventions in experimental researches. (J Vasc Surg 2009;50:1423-32.)**

Clinical Relevance: **Abdominal aortic aneurysm (AAA) typically has a silent nature, and its rupture has high morbidity and mortality. There are currently no therapeutic approaches to prevent AAA, and complete mechanisms of AAA formation are still poorly understood. We developed a novel rat AAA model using a combination of intraluminal elastase infusion and extraluminal calcium chloride exposure. This model is simple and easy to perform and is highly reliable and reproducible to create a saccular aneurysm. It could become a powerful tool not only to elucidate etiopathogenetic mechanisms of AAA formation but also to explore new diagnostic and therapeutic possibilities.**

Abdominal aortic aneurysm (AAA) is a degenerative disease characterized by destruction of aortic architecture and subsequent dilatation. Because of its silent nature, the likely sequelae of undiagnosed AAAs include rupture, which can be fatal and requires emergency surgical management. Although the main pathogenesis of AAAs is atherosclerosis, multiple environmental and genetic risk factors are considered to be involved in aneurysm formation and progression.[1] Previous research has shown that various inflammatory networks contribute to the AAA formation through certain signaling pathways to promote the degradation of extracellular matrix or impair the biosynthesis of extracellular matrix,[2-6] but complete mechanisms of AAA formation are still poorly understood. Therefore, establishing experimental models for the AAA research should be of critical importance.

Several animal models have been developed to assist in our understanding of the pathophysiologic mechanisms of AAA formation. One of the most popular is the elastase-induced model of AAA that was first introduced by Anidjar et al.[7] The concept for the AAA development is based on the disrupted nature of elastin, which consists of extracellular matrix in aortic walls.

Although many reports proved the efficacy of elastase,[8-12] Carsten et al[13] indicated that this model requires a

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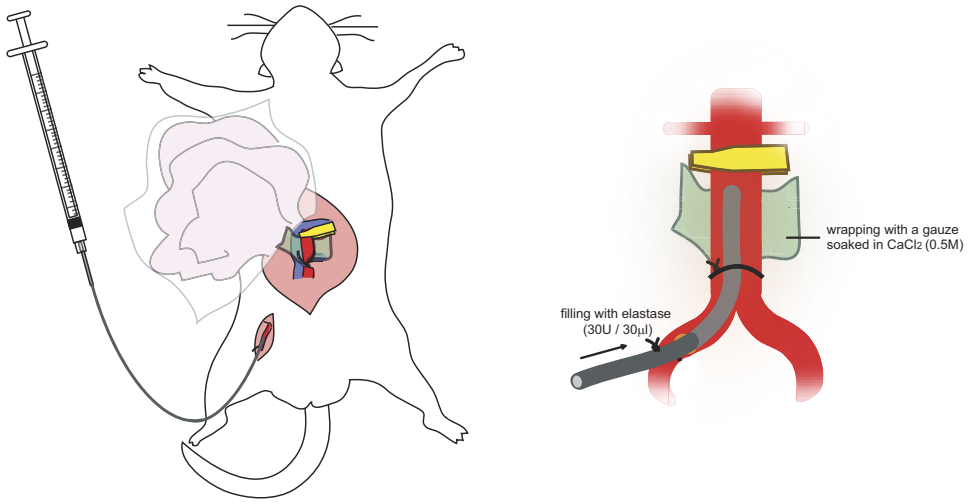

Fig 1. A schematic drawing shows the rat abdominal aortic aneurysm model using a combination of intraluminal elastase-infusion and extraluminal calcium chloride exposure.

long infusion time (2 hours) of elastase to create AAAs, which may result in high mortality due to an extensive aortic elastolysis and a long hind limb ischemia. Recently, Yamaguchi et al[14] and Sinha et al[15,16] have shown successful rat AAA models with a short elastase infusion time of 30 minutes.

Another inflammatory modifier might be helpful in the elastase-induced model to create reliable AAAs that simulate human aneurysms. Another popular model is the calcium chloride ($CaCl_2$)-induced AAA model that was first reported by Gertz et al.[17] Periaortic $CaCl_2$ application promotes inflammatory responses, followed by structural disruption of the aortic walls that leads to the AAA development.[2] This model, however, does not always result in sufficient aneurysmal formation. Isenburg et al[18] have reported that its dilatation rate is only 66.7%, even with an arbitrary threshold of a 20% diameter increase considered as an aneurysm.

The purposes of this study were to introduce a new rat AAA model using a combination of intraluminal elastase infusion and extraluminal $CaCl_2$ exposure, with a detailed description of surgical techniques and to evaluate the reliability and reproducibility of the AAA model compared with AAA models with a single application of elastase or $CaCl_2$.

MATERIALS AND METHODS

Animals. This study used 71 male Sprague-Dawley rats (8 weeks old, CLEA Japan Inc, Tokyo). The handling of the rats and their use in experiments conformed to the *Guidelines for Animal Experiment* at Kobe University Graduate School of Medicine and the *Guide for the Care and Use of Laboratory Animals* published by the National Academy Press.[19]

Anesthesia. Rats were placed in a closed chamber containing diethyl ether (Wako, Osaka, Japan). After loss of consciousness, the rats were anesthetized by the intraperitoneal administration of pentobarbiturate (1 mg/kg).

A new AAA model. Anesthetized rats were placed under an operating microscope Leica M651 (Leica Microsystems, Heerbrugg, Switzerland; ×6 to ×10 magnification). A long midline abdominal incision from pubis to xiphoid was made, and the abdominal cavity was exposed. The intestines were retracted superiorly and covered with wet gauze to keep them moist during the operation.

The abdominal aorta was exposed below the renal vessels. All lumbar branches of the exposed infrarenal aorta were ligated. Through a groin incision, a SP10 polyethylene catheter (Natsume Seisakusho, Tokyo, Japan) was inserted into a right femoral artery and advanced in the infrarenal aorta. An atraumatic microvascular clamp was placed on the aorta just below the left renal vein, and a temporary ligature with 4-0 silk was placed 1 cm distal to the clamp.

The isolated region of the aorta was filled with 30 μL (30 U) of porcine pancreatic elastase (135 U/mg; Elastin Products, Mo) through the SP10 catheter without any aortic expansion, and gauze soaked in 0.5M $CaCl_2$ (Sigma-Aldrich, Tokyo, Japan) was wrapped the aorta circumferentially. After 20 minutes of intraluminal static elastase infusion and extraluminal $CaCl_2$ exposure, the catheter was removed and the right femoral artery was ligated. The microvascular clamp and the temporary ligature were removed from the aorta to restore blood flow.

The intestines were returned to the abdominal cavity. The abdominal incision was closed with continuous running 5-0 nylon suture, and the rats were placed in warming cages to recover. The schematic drawing of this AAA model is shown in Fig 1.

Experimental groups. Rats were divided into the three groups at random according to their treatment allocation as follows: 26 to intraluminal elastase infusion and extraluminal $CaCl_2$ exposure (group EC), 24 to intraluminal elastase infusion and extraluminal vehicle (saline) exposure (group E), and 21 to intraluminal vehicle (saline) infusion and extraluminal $CaCl_2$ exposure (group C). Except for their treatment allocation, there were no procedural differences among the three groups.

The end points of the present study were 1 and 4 weeks after treatment in each group. The 1-week samples were used for morphometric and molecular analyses (6 in each group) and immunohistochemical analysis (6 in each group). The 4-week samples were used for morphometric and histologic analyses (14 in group EC, 12 in group E, and 9 in group C).

Macroscopic assessments. Animals were anesthetized, and then the abdominal aorta was exposed and photographed with a digital camera (Leica IC D, Leica Microsystems). The aneurysmal diameter, defined as the maximal dimension of the transverse minor axis of the abdominal aorta, and the aortic diameter at the infrarenal proximal neck site, were measured under physiologic conditions with an optical micrometer by a person (Z. C.) who was blinded to the different treatment groups. The dilation ratio was calculated according to the following formula: Dilation ratio (%) = [(aneurysmal diameter − aortic diameter)/aortic diameter] × 100. The AAA is defined as when the dilation ratio is >50%.[20]

Specimen preparation. The treated 1-cm aortic segment in each group was harvested at its study end point. The 1-week aortic segments for molecular analysis were cut into two equal parts transversely. One segment was used for messenger RNA (mRNA) analysis, and the remaining part was used for gelatin zymographic analysis. The 1-week aortic segments for immunohistochemical analysis were embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek Japan Co, Tokyo, Japan) in liquid nitrogen and stored at −80°C until needed. The 4-week aortic segments for histologic analysis were fixed in 10% formalin, dehydrated in a graded ethanol bath, cleaned in xylene, and embedded in paraffin.

Histology. Paraffin-embedded 5-μm-thick sections were stained by hematoxylin and eosin (HE) for general appearance, elastin von Gieson (EVG) for elastin, and picrosirius red (PSR) for collagen. Images for the sections were captured with a microscopic system (BZ-8100, KEYENCE Co, Osaka, Japan) and assessed by using ImageJ 1.41 software (National Institute of Health, Bethesda, MD).

The thickness of aortic wall was measured from the average thickness at 8 points of the cross-sectional aortic wall in the HE-stained sections. Areas of elastin and collagen in a cross-sectional aortic wall were semiquantified using the ImageJ plug-in Color Counter with a color threshold mask. Elastin content was calculated by dividing the elastin-positive area by the cross-sectional media area of the aortic wall, which was measured using the EVG-stained sections to highlight the internal elastic lamina and external elastic lamina. Collagen content was calculated by dividing the collagen-positive area by the cross-sectional aortic wall area in the PSR-stained sections. Elastin and collagen contents were expressed as a percentage.

Immunohistochemistry. Immunohistochemical staining was performed on frozen sections (5 μm) by using anti-immunoglobulin horseradish peroxidase detection kits (BD Pharmingen, San Diego, CA) with primary antibodies for T-lymphocyte and leukocyte markers (mouse anti-rat CD3 and CD11b, respectively; BD Pharmingen). Diaminobenzidine substrate was used as a chromogen, and cell nuclei were counterstained with hematoxylin. Positive cell numbers were quantified by counting reactive cells in five nonoverlapping high-power fields using the ImageJ software.

Quantitative real-time polymerase chain reaction analysis. Total RNA was isolated from graft samples using an RNeasy fibrous tissue mini-kit (Qiagen, Valencia, Calif) according to the manufacturer's instructions. The RNA was transcribed and amplified to complimentary DNA (cDNA) using a high-capacity cDNA reverse-transcription kit (Applied Biosystems, Foster City, Calif). Quantitative real-time polymerase chain reaction (PCR) analysis for mRNA of matrix metalloproteinases (MMP)-2, MMP-9, tumor necrosis factor (TNF)-α and interleukin (IL)-1β was performed using ABI Prism 7500 sequence detector system (Applied Biosystems) with TaqMan universal PCR master mix and TaqMan real-time PCR primers (Applied Biosystems). The expression level of each mRNA was divided by mRNA level of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Gelatin zymography. Proteins from aortic specimens were extracted using a buffer containing 50mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.2% sodium dodecyl sulfate, and 1mM ethylenediaminetetraacetic acid, supplemented with protease inhibitors (20 μg/mL aprotinin, 10 μg/mL leupeptin, and 1mM phenylmethylsulfonyl fluoride). To determine gelatinolytic activities of MMP-2 and MMP-9 in the treated aorta, gelatin-zymography kit (Primary Cell Co, Hokkaido, Japan) was used according to the manufacturer's instructions. The protein concentration was standardized with a microbicinchoninic acid protein assay kit (Pierce, Rockford, Ill), and then protein (20 μg) was applied in each lane for the electrophoresis. Densitometric analysis of the lytic bands was performed by ImageJ software.

Statistical analysis. Database management and statistical analysis were performed with StatView 5.0 software (SAS Institute Inc, Cary, NC). All values are expressed as means ± standard error of the mean for the number of rats. Comparisons among the three groups were performed using analysis of variance with Bonferroni correction method. Values of $P < .05$ were considered statistically significant.

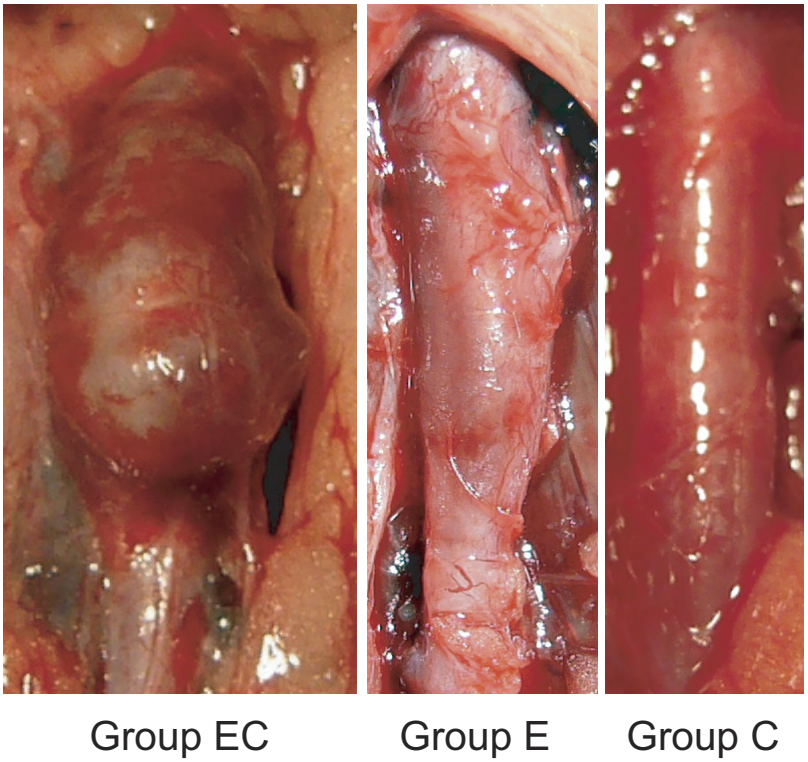

Fig 2. Photographs show aneurysmal lesions of abdominal aorta at 4 weeks in each group. *Group EC,* Combined elastase and calcium chloride; *group E,* elastase; *group C,* calcium chloride.

Table. Macroscopic assessment of abdominal aortic aneurysms

	AAA formation, No. (%)		*Dilation ratio, %*	
Group	*1 week*	*4 weeks*	*1 week*	*4 weeks*
Group EC	3/6 (50)[a]	13/14 (92.7)[a]	51.8 ± 3.5[a]	94.8 ± 9.9[a]
Group E	1/6 (16.7)	3/12 (25.0)	22.4 ± 7.2	43.3 ± 6.3
Group C	0/6 (0)	0/9 (0)	3.5 ± 3.5	10.9 ± 4.2

C, Calcium chloride; *E,* endothelin; *EC,* elastase and calcium chloride combined.
[a]$P < .001$ vs group E or Group C.

RESULTS

The surgical procedure in each group required approximately 30 minutes to complete. No technical failures or deaths occurred intraoperatively. No thrombosis formation at the infrarenal aorta was observed postoperatively, and all animals survived until each end-point uneventfully.

Macroscopic assessments. Aneurysm formation was observed at 1 and 4 weeks. Macroscopic findings are shown in Fig 2 and the Table. According to AAA definition in the present study, an AAA had already formed in 50% of the animals in group EC at 1 week, and 92.7% had saccular AAAs at 4 weeks. No AAAs formed in group C, and only 25% of animals in group E showed AAAs at 4 weeks. There were significant differences of the AAA formation at 1 and 4 weeks in group EC compared with group E or C. The dilation ratios of the AAAs at 1 and 4 weeks were significantly bigger in group EC than those in group E or C.

Histology. Histology of the treated aorta at 4 weeks was assessed to evaluate aneurysm formation in each group (Fig 3). The aortic wall thickness was 125.4 ± 5.6 μm in group EC, 149.6 ± 6.5 μm in group E, and 152.9 ± 7.2 μm in group C. The treated aorta in group EC was dilated with a significantly thinner wall compared with group E or C. EVG staining showed that degeneration and disruption of the elastic lamellae were particularly observed in group EC. Elastin content in group EC was significantly less than that in group C, although the difference between group EC and E was not significant. PSR staining showed no significant differences of collagen content among the three groups.

Inflammatory response. Inflammation is believed to be part of the etiology in AAA formation by direct local production of proinflammatory cytokines such as TNF-α and IL-1β, and MMPs such as MMP-2 and MMP-9.[2] At 1 week, mRNA expression of TNF-α, IL-1β, MMP-2, and MMP-9 in the treated aorta was significantly upregulated in group EC compared with group E or C (Fig 4, *A*). Gelatinolytic activities of MMP-2 and MMP-9 proteins in the

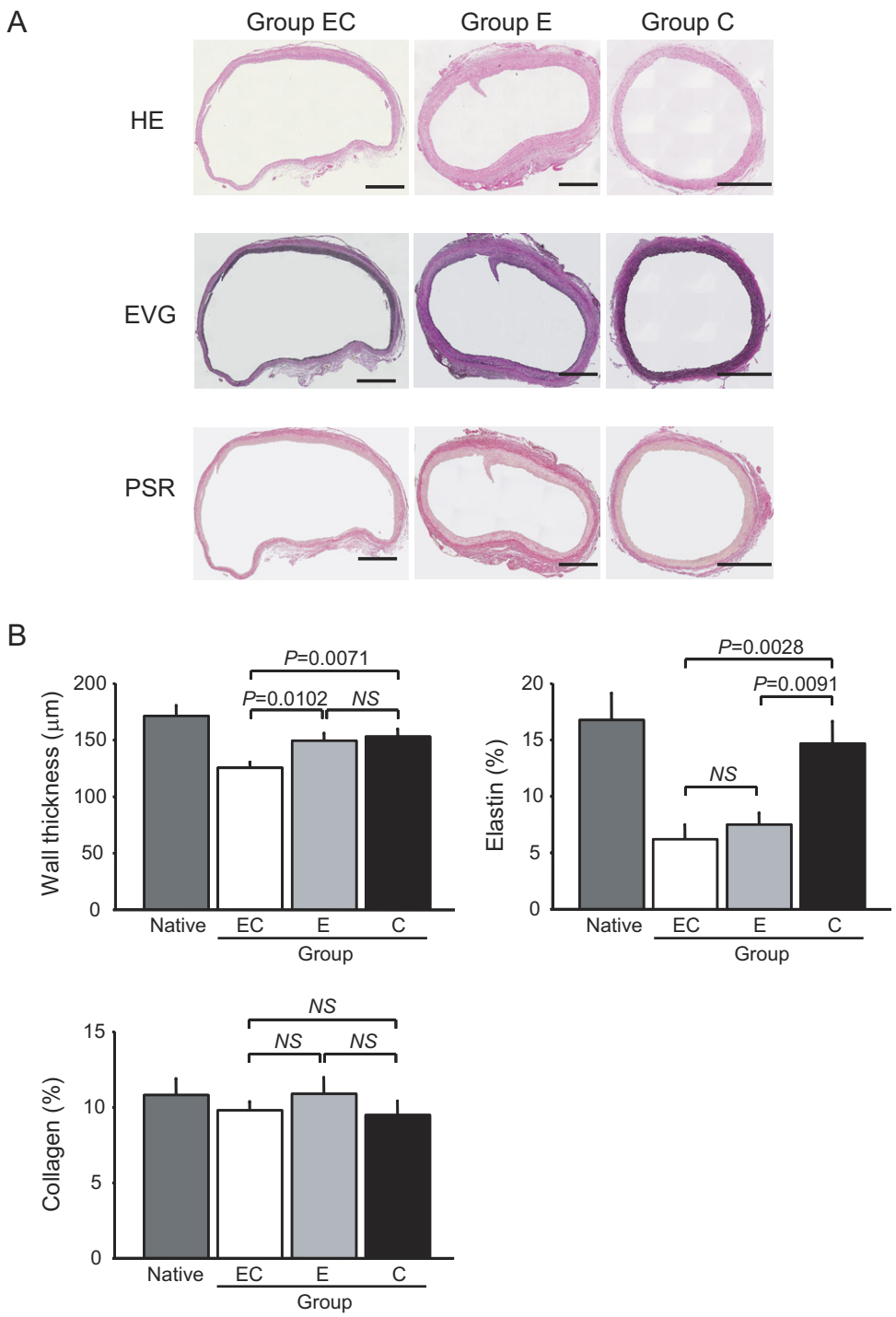

Fig 3. A, Histologic findings of abdominal aortic aneurysms (AAAs) are shown at 4 weeks after staining with hematoxylin and eosin *(HE)* for an assessment of aortic wall thickness, elastin von Gieson *(EVG)* staining for an assessment of elastin content, and picrosirius red *(PSR)* staining for an assessment of collagen content in group EC. Bar, 300 μm. **B,** Quantitative analysis is shown for the three variables. *NS,* Not significant. All data are expressed as mean ± standard error for 14 mice in group EC (elastin and calcium chloride combined), 12 in group E (elastin only), and 9 in group C (calcium chloride only).

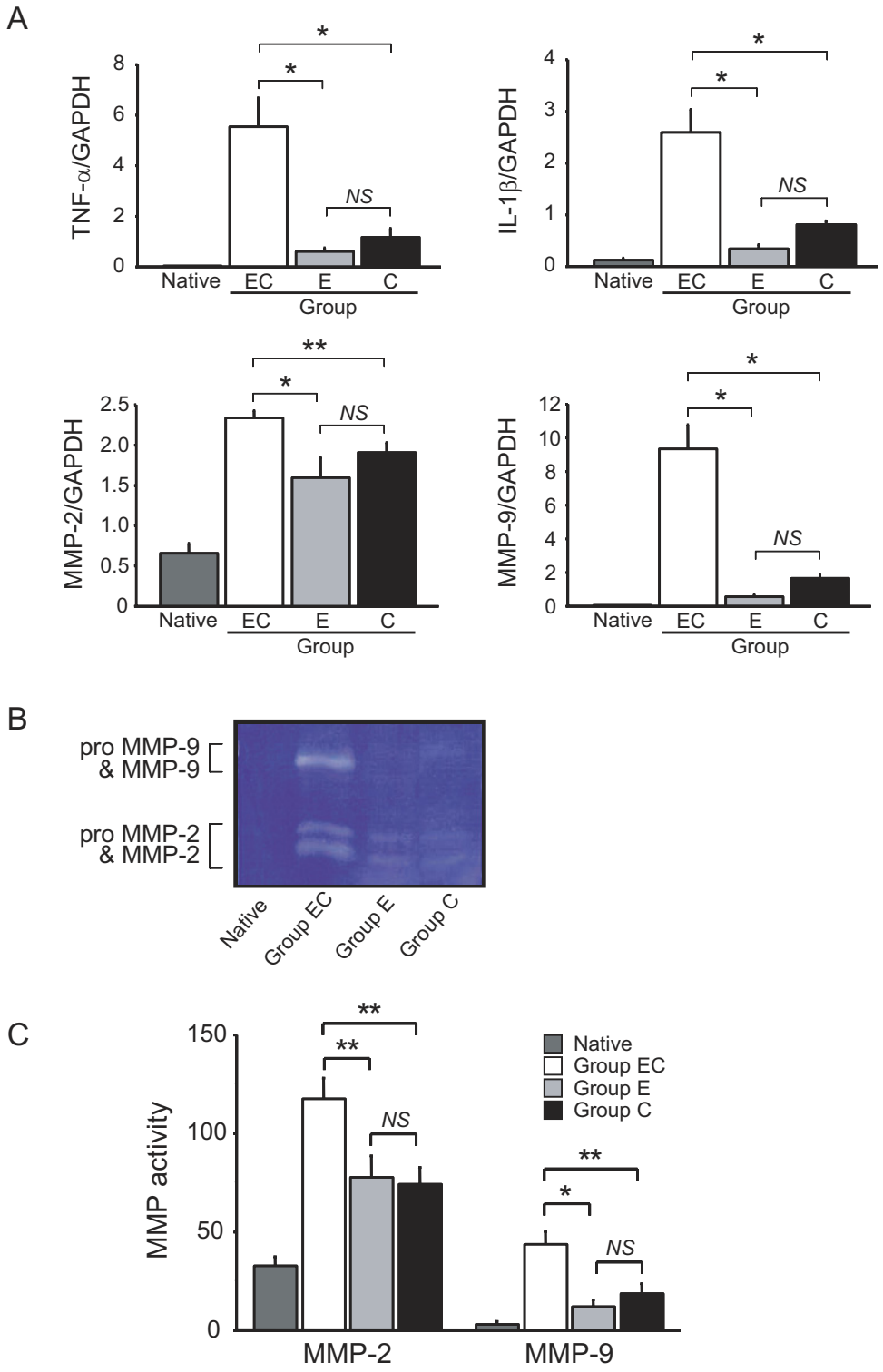

Fig 4. Protease activity and messenger RNA (mRNA) expression in abdominal aortic aneurysms at 1 week **A,** Intra-aortic mRNA expression of tumor necrosis factor (*TNF*)-α, interleukin (*IL*)-1β, and matrix metalloproteinase (*MMP*)-2 and MMP-9. **B,** Gelatin zymography documents gelatinolytic activities of MMP-2 and MMP-9. **C,** Densitometric analysis of the MMP activities. $*P < .0001$, $**P < 0.01$. *NS,* Not significant. All data are expressed as mean ± standard error of the mean for 6 rats per group.

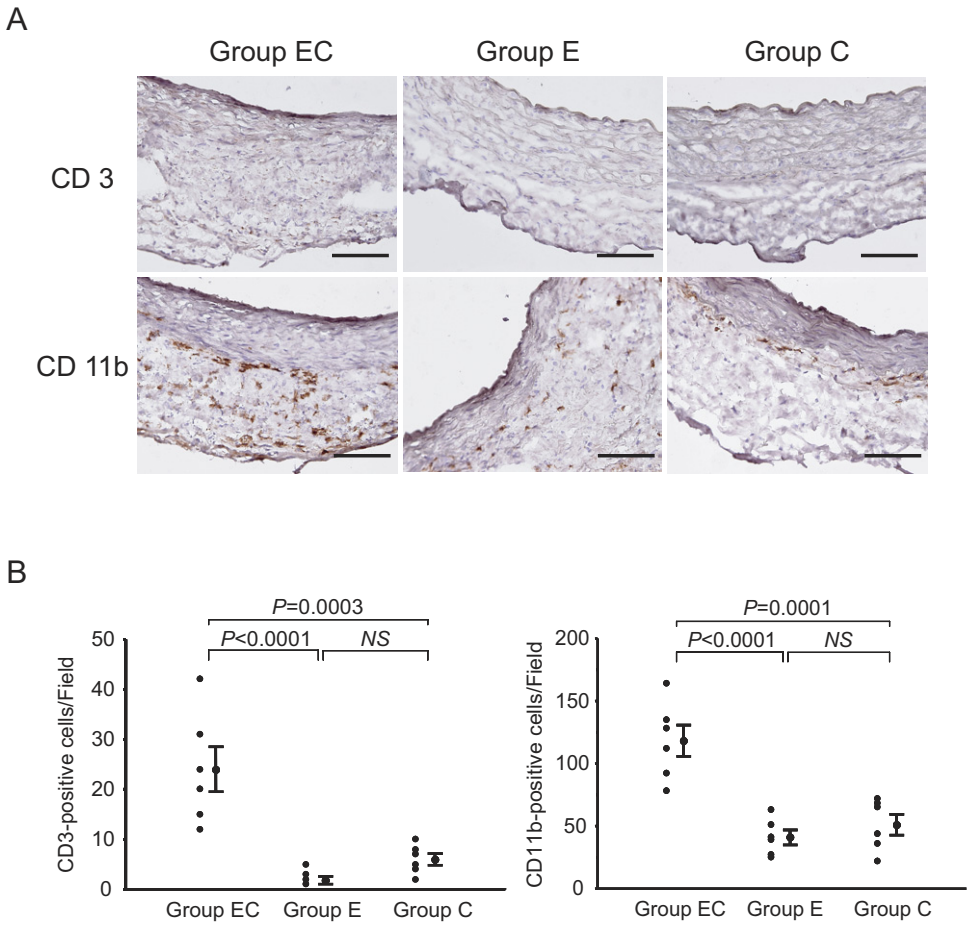

Fig 5. Inflammatory cell infiltration in abdominal aortic aneurysms at 1 week. **A,** Immunohistochemical staining of CD3 and CD11b. Bar, 100 μm. **B,** Quantitative analysis of CD3+ and CD11b+ cells. *NS,* Not significant. All data are expressed as mean ± standard error of the mean for 6 rats per group.

treated aorta were significantly higher in group EC than in group E or C (Figs 4, *B* and *C*).

Immunohistochemistry. To observe an infiltration of inflammatory cells in the treated aorta at 1 week, immunohistochemical stainings with T-lymphocyte marker (anti-CD3) and leukocyte marker (anti-CD11b) were performed. T-lymphocytes and leukocytes tended to infiltrate in the adventitia and outer media of the treated aortas in all groups. The numbers of infiltrating CD3+ and CD11b+ cells were significantly increased in group EC compared with group E or C (Fig 5).

DISCUSSION

This study detailed a new method for creating AAAs using a combination of intraluminal elastase infusion and extraluminal $CaCl_2$ exposure in rats and showed that this is a reliable and reproducible saccular aneurysm model with (1) dilated and thin aortic walls, (2) increased degradation of elastin, (3) upregulated expressions and activities of MMP-2 and MMP-9, and (4) enhanced inflammatory responses. In experienced hands, the surgical procedure only requires about 30 minutes to complete.

The elastase-induced AAA model is a standard aneurysm model for in vivo research in small animals.[8-12] Elastase breaks down elastin, which determines the structural and mechanical properties of aortic extracellular matrix. Anidjar et al,[7] who first established the elastase-induced experimental aneurysm model in rats, suggested that the main involvement of elastase in the aneurysm formation is through enhanced elastolytic activity and a loss of elastin in the aortic walls. Although this initial report did not clearly mention the elastase infusion time, most laboratories have applied a 2-hour infusion time to create the AAAs. Some investigators have recently pointed out that the 2-hour infusion time in this model has some drawbacks, including high mortality related to the long elastase infusion time and hind limb ischemia caused by the cross-clamping period of the infrarenal aorta.[13,21] We also recognized the difficulty in keeping a constant pressure for the elastase infusion for such a long time.

A decrease in the elastase infusion time would be expected to reduce those drawbacks, and Yamaguchi et al[14] and Sinha et al[15,16] have recently shown successful rat AAA models with a 30-minute elastase infusion. Although their procedures to form AAAs were similar to our AAA models, the 1- to 2-mL volume of infused elastase solution in their models was quite different from the 30-μL amount in our models. Our previous trial of the 1-mL elastase infusion showed that the infrarenal aorta was extremely expanded, followed by a burst of ligated lumbal branches or a rupture of the aorta itself. Because human AAAs would not be created by such a mechanical pressure stress, our model was designed so that elastase was statically infused into the clamped infrarenal aorta without any expansion. However, our data showed that only 25% of animals with the 20-minute elastase infusion (group E) showed successful AAAs at 4 weeks. Carsten et al[13] have suggested that some other inflammatory modifier might be needed in the elastase-induced model to create reliable AAAs that simulate human aneurysms.

An AAA model induced by $CaCl_2$ is another standard aneurysm model in small animals.[2,17,22,23] An extraluminal exposure of 0.1 to 0.5M $CaCl_2$ was usual in most laboratories. The chemical damage that occurs when the outer surface of aorta is exposed to $CaCl_2$ promotes inflammatory and arteriosclerotic reaction on the adventitia and subsequent aortic dilatation.[17] A high concentration of $CaCl_2$ produces tissue injury by an exothermic reaction around the tissue that depends on the amount, concentration, and the duration of exposure.[24] Freestone et al[25] previously demonstrated a dose-dependent $CaCl_2$-induced aortic injury at concentrations of 0.01 to 0.5M.

In our preliminary evaluation of the viability of aortic tissues with $CaCl_2$ exposure ex vivo by using the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan), the difference of the viability of aortic tissues between 0.5M $CaCl_2$ exposure and vehicle exposure was not significant (data not shown). This result suggested that extraluminal 0.5M $CaCl_2$ exposure does not cause cell death resulting in aneurysmal dilation. Although mild tissue adhesion around the $CaCl_2$-treated aorta was observed, no changes were observed in the adjacent inferior vena cava such as vascular sclerosis or dilation. In the present study, tissue injury with inflammatory cell infiltration and proinflammatory cytokine activation in the aortic wall was more severe in the $CaCl_2$-induced model than in the elastase-induced model. Nevertheless, there was no AAA formation with an arbitrary threshold of a 50% diameter increase in the $CaCl_2$-induced model (group C). Some other factors might be needed in the $CaCl_2$-induced model to create reliable AAAs.

In the AAA model in our study (group EC), elastase is applied intraluminally and $CaCl_2$ extraluminally. The short incubation period of 20 minutes achieved sufficient aneurysmal dilatation, with a high success rate of saccular AAA formation at 4 weeks. Although most AAAs found in human pathology are fusiform, saccular AAAs are thought to be more prone to expand and rupture than fusiform aneurysms. Because the saccular AAAs were not created in groups C or E, we do not think that a portion of the infrarenal rat aorta is more susceptible to structural wall changes or that the formation of the saccular component of the aneurysm has anything to do with the polyethylene catheter or clamp/tie.

The reasons why elastase in the presence of $CaCl_2$ produces mainly saccular aneurysms are assumed to be a synergistic effect of elastase and $CaCl_2$ that stimulates an inflammatory response and an elastolytic cascade in the aortic walls. Recent studies suggest that adventitial inflammation in the aortic wall, in addition to wall elastolysis, could have a detrimental role in AAA formation.[23,26-28] Aortic adventitial injury due to $CaCl_2$ induces the accumulation and activation of inflammatory cells such as leukocytes and T lymphocytes. Recruitment of inflammatory cells into the aortic walls be an important source of proinflammatory cytokines such as TNF-α and IL-1β and contribute to the aneurysmal growth and rupture with subsequent elaboration of MMP-2 and MMP-9.[2,29] MMP upregulation due to $CaCl_2$ as well as elastase could enhance aortic elastin degradation synergistically. Actually, the present study showed more infiltration of leukocytes and T lymphocytes in the adventitia, more upregulation of TNF-α, IL-1β, MMP-2, and MMP-9, and less elastin content in group EC, leading to progressive AAA formation.

Elastase is a member of a family of serine proteases that stimulate protease-activated receptors in aortic smooth muscle cell, leading to the inhibition of Ca^{2+} influx that is necessary for vascular contraction.[30,31] This inhibition of smooth muscle contraction may be one of factors for aortic dilation. Furthermore, although a low concentration of $CaCl_2$ is a key component in Krebs solution and physiologic smooth muscle contractions, a high concentration of $CaCl_2$ might induce smooth muscle injury and then inhibit its contractions, resulting in aneurysmal formation. The subsequent circumferential aortic dilation would further enhance both elastase-induced elastolysis and $CaCl_2$-induced injury in the aortic walls.

In the clinical settings, aneurysms typically have no signs or symptoms, and AAA rupture has high morbidity and mortality. There are currently no therapeutic approaches to prevent AAA, leaving patients with surgical or endovascular therapy as their only option. Experimental aneurysm modeling could become a powerful tool to elucidate the cellular and molecular mechanisms of the AAA pathogenesis. Targeting biologic pathways in AAA pathogenesis may benefit patients by slowing AAA growth and possibly preventing rupture. For these purposes, it is important to have a reliable and reproducible animal model for AAAs that simulates true human aneurysms. The attractive features of our model are a high success rate and optimized characteristics for aneurysm formation. Our technique is also simpler and easier to perform, with a short preparation time. We firmly believe that this new approach to produce an animal AAA model can bring a better understanding of the mechanisms that contribute to aneurysm

formation and then allow us more promising approaches to prevent or treat AAA diseases.

This study has some limitations: The first is that the present AAA model lacks several prominent features of the human lesion such as atherosclerosis and intraluminal thrombosis.

Second, the present control AAA models induced by elastase or $CaCl_2$ are not completely similar to the conventional AAA models in regard to dosage and incubation period. We did, however, perform similar treatment conditions among the study groups to compare the efficacy of our new AAA model.

Third, we did not compare this model with other AAA models such as angiotensin-associated, genetically predisposed, or hemodynamically induced aneurysms[32] because of their mechanistic differences of aneurysmal formation, or with mouse AAA models because of their species difference.

Finally, the present study does not exclude the possibility that subsets of collagen types, such as type I or type III collagen, may be affected by elastase or $CaCl_2$ application, potentially leading to aortic structural changes and AAA formation.

CONCLUSIONS

The novel rat AAA model we have developed using a combination of intraluminal elastase infusion and extraluminal $CaCl_2$ exposure has similarities to the human pathophysiology of aneurysm formation. This model is simple and easy to perform and is highly reliable and reproducible to create a saccular aneurysm. We believe that our AAA model is an ideal model that can be well used not only for further research into etiopathogenetic processes leading to the AAA development but also for the study of new diagnostic and therapeutic possibilities.

AUTHOR CONTRIBUTIONS

Conception and design: AT, TH, KO
Analysis and interpretation: AT, TH, KO
Data collection: AT, TH, ZC
Writing the article: AT, TH
Critical revision of the article: TH, YO, KO
Final approval of the article: YO, KO
Statistical analysis: AT, TH
Obtained funding: YO, KO
Overall responsibility: KO
AT and TH contributed equally to this work

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