Dietary DNA Attenuates the Degradation of Elastin Fibers in the Aortic Wall in Nicotine-Administered Mice

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Summary Abdominal aortic aneurysm (AAA) is a vascular disease characterized by chronic inflammation in the infrarenal aorta. Epidemiologic data have clearly linked tobacco smoking to aneurysm formation and a faster rate of expansion. It suggested that nicotine, one of the main ingredients of tobacco, has been suggested to be associated with AAA development and rupture. In the condition where no established drugs are available; therefore, an effective approach to prevent the vascular damage from nicotine consumption may be the use of dietary functional food factors. However, little is known about the relationship between dietary components and AAA. In this study, we estimated the effect of dietary deoxyribonucleic acid (DNA) on the vascular wall. After habituation for 5 d, the mice were divided into four groups: control diet and distilled water group (C), DNA-Na diet and distilled water group (DNA), control diet and 0.5 mg/mL nicotine solution group (C-Nic), DNA-Na diet, and 0.5 mg/mL nicotine solution group (DNA-Nic). The dietary DNA attenuated the degradation of elastin fibers induced by nicotine administration. The areas stained positive for MMP-2 in the DNA-Nic group were significantly suppressed compared to C-Nic mice. These data suggest that the dietary DNA may prevent the weakening of the aortic wall via inhibition of the MMP-2-dependent pathway. In conclusion, we have revealed the protective effect of dietary DNA on the vascular pathology of nicotine-administered mice. A nucleic acid-rich diet might be useful for people who consume nicotine via smoking, chewing tobacco, or nicotine patches.

Key Words abdominal aortic aneurysm, nucleic acid, dietary DNA, matrix metalloproteinase, nicotine

Abdominal aortic aneurysm (AAA) is a circulatory system disease characterized by aorta dilation with a sudden rupture. Characteristics such as male sex, older age, smoking status, dyslipidemia, and hypertension are all associated with AAA (1, 2). Patients with an increased risk of rupture have no choice but to undergo surgery either by open repair with prosthetic graft replacement or endovascular stent graft placement (3). It has been reported that cigarette smoking is a major risk factor for AAA formation (4), and many AAA patients have a smoking history (5). Epidemiologic data have clearly linked tobacco smoking to aneurysm formation and a faster rate of expansion (6, 7).

Recently, it was suggested that nicotine, one of the main ingredients of tobacco, was associated with AAA development and/or rupture. Wang et al. reported that nicotine increases aneurysm formation by increasing intracellular oxidative stress and cytokines, which promote the activation and nuclear translocation of AMPKα2 in vascular smooth muscle cells (VSMCs) (8, 9). Additionally, Maegdefessel et al. reported that AAA formation by nicotine is related to the expression of microRNA-21 (10). We have previously reported that increases in oxidative stress, MMP activity and the degradation of vascular wall fiber content are facilitated by nicotine in both mice and rats (11, 12). These studies suggested a direct association between nicotine and AAA-related factors. In addition to smoking, the consumption of other forms of nicotine such as chewing tobacco or nicotine patches, which are used for smoking cessation, might be a potential risk for AAA development.

There are no established drugs available for this condition; therefore, functional food factors may provide an effective approach to preventing vascular damage from nicotine consumption. We previously demonstrated that dietary fish oil suppressed the degradation of elastin fibers in nicotine-administered mice (12). In addition, n-3 polyunsaturated fatty acids reportedly

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Table 1. Effect of DNA on physiological variables and serum biochemistry. Data are expressed as the mean±SE.

| Variable                        | C          | DNA        | C-Nic      | DNA-Nic    |
|---------------------------------|------------|------------|------------|------------|
| Initial body weight (g)         | 16.24±0.68 | 16.28±0.54 | 16.22±0.54 | 16.20±0.41 |
| Final body weight (g)           | 22.21±0.38 | 21.41±0.49 | 21.09±0.32 | 20.45±0.26 |
| Food intake (g)                 | 5.74±0.04  | 5.7±0.13   | 5.63±0.05  | 5.47±0.04  |
| Water intake (g)                | 3.61±0.14  | 3.63±0.23  | 2.80±0.15  | 2.63±0.10  |
| Liver weight (g/100 g body weight) | 5.62±0.17 | 5.52±0.14  | 4.88±0.09  | 5.03±0.12  |
| Kidney weight (g/100 g body weight) | 1.40±0.07 | 1.49±0.04  | 1.47±0.03  | 1.54±0.06  |
| Spleen weight (g/100 g body weight) | 0.31±0.02 | 0.32±0.04  | 0.36±0.06  | 0.39±0.04  |
| Heart weight (g/100 g body weight) | 0.51±0.04 | 0.53±0.03  | 0.53±0.02  | 0.56±0.03  |
| Peritesticular fat weight (g/100 g body weight) | 1.41±0.11 | 1.25±0.09  | 1.16±0.10  | 1.24±0.05  |
| Serum glucose (mg/dL)           | 343.40±12.68 | 373.30±11.38 | 321.53±19.25 | 312.19±24.24 |
| Serum triglyceride (mg/dL)      | 81.50±3.97 | 71.40±8.07 | 74.75±8.15 | 85.33±8.17 |
| Serum total cholesterol (mg/dL) | 119.76±4.14 | 121.61±2.97 | 123.95±3.33 | 114.16±4.60 |

Different letters mean significant difference.

attenuate the development and rupture of AAA by multiple mechanisms in several experimental animals (13, 14). It is desirable to increase the number of functional food factor options that will allow dietary components to prevent AAA, so that a balanced diet can be maintained. However, little is known about the relationship between dietary components and AAA. In this study, we focused on the nucleotide-rich diet. In the human body, rapid proliferating systems such as immune cells or small intestines utilize exogenous nucleotides because they are not able to fulfill their nucleotide needs by de novo synthesis (15). In human clinical trials, dietary nucleotide supplementation improves irritable bowel syndrome (16). It has also been reported that dietary nucleotide supplementation has other beneficial effects such as improvement of oxidative stress in the liver of alcohol-treated rats (17) and enhancement of immunocompetence (18–20). Maldonado et al. reported that dietary nucleotides may be semi-essential under certain circumstances such as disease, poor diet or stress (21). Nicotine consumption induces an immune response in the vascular wall (11, 12); therefore, it is of interest to estimate the effect of dietary nucleotides on the vascular wall. Here, we show that dietary deoxyribonucleic acid (DNA) attenuates the nicotine-induced weakening of the mouse aortic wall.

MATERIALS AND METHODS

Materials. Nicotine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Nicotine was dissolved in distilled water (nicotine solution). DNA sodium salt (DNA-Na) was a gift from LS Corporation (Tokyo, Japan). The DNA-Na was purified from salmon milt and the contents of DNA-Na specification was more than 86% (w/w) by HPLC analysis.

Animals. All animal experiments were approved by the Kindai University Animal Care and Use Committee and performed according to the Kindai University Animal Experimentation Regulations (Approval number: KAAG-25-002). Three-week-old male C57BL/6J mice (Japan SLC, Inc., Shizuoka, Japan) were provided with food and water ad libitum, and housed in a humidity-controlled room with a 12-h light and dark cycle. Room temperature was maintained at 25±1°C. After habituation for 5 d, the mice were divided into four groups: control diet and distilled water group (C), DNA-Na diet and distilled water group (DNA), control diet and 0.5 mg/mL nicotine solution group (C-Nic), DNA-Na diet and 0.5 mg/mL nicotine solution group (DNA-Nic). Each respective diet was administered to the C (n=6), the DNA (n=6), the C-Nic (n=8) and the DNA-Nic (n=9). After 2 wk, all mice were sacrificed. The diet composition is shown in Table S1 (Supplemental Online Materials).

Sample collection. The abdominal aorta was isolated. The isolated tissue was fixed in 4% paraformaldehyde (PFA) (Nacalai Tesque, Inc., Kyoto, Japan), soaked in order of different percentages of ethanol (70%, 80%, 90% and 100%) and xylene, and then embedded in paraffin (Sakura Finetek Japan, Tokyo, Japan).

Histological analysis. Cross-sections of isolated aorta (4 µm thickness) were prepared using a microtome (Yamato Kohki Industrial, Saitama, Japan) and mounted on glass slides. The aortic walls were stained with hematoxylin–eosin (HE), Elastica van Gieson (EVG), Picrosirius red (PSR), and other immunohistochemical stains. Quantitative analyses of the histological stains were performed using ImageJ software (National Institutes of Health, Bethesda, Maryland). The destruction rate of the wavy configuration of the elastic lamina was calculated by dividing the area of destruction (indicated by flattening and fragmentation of the elastic lamina) by the entire area of elastic lamina.

Immunohistochemical staining. The tissue sections were deparaffinized, rinsed in phosphate-buffered saline...
Dietary DNA Attenuates the Degradation of Elastin Fiber

PBS) with 1% Triton-X 100, and then incubated in 10% oxalic acid for 1 h. For antigen activation, 0.1% trypsin in PBS was added to the tissue sections. Endogenous horseradish peroxidase (HRP) in the tissue sections was blocked using 3% aqueous hydrogen peroxide in methanol for 8 min. After being washed in PBS, the tissue sections were blocked with Blocking One Histo (Nacalai Tesque). The following primary antibodies were used to histologically assess the aortic wall: rabbit anti-MMP-2 (1 : 100; Thermo Scientific, San Jose, CA), goat anti-MMP-9 (1 : 100; Santa Cruz Biotechnology, Dallas, TX), rabbit anti-MMP-12 (1 : 100; Bioss Antibodies, Woburn, MA), mouse anti-malondialdehyde (MDA) (1 : 100; Abcam, Tokyo, Japan), rabbit anti-macrophage chemotactic protein-1 (MCP-1) (1 : 100; Bioss Antibodies) and rabbit anti-CD68 (1 : 100; Bioss Antibodies). The sections were incubated with the appropriate primary antibody overnight at 4˚C. On the following day, the sections were rinsed in PBS, and incubated with the appropriate secondary antibody conjugated to HRP. Slides were developed with DAB (Vector Laboratories, Burlingame, CA), dehydrated in order of different percentages of ethanol (80%, 90%, and 100%), cleared in xylene, and covered with lipid-soluble mounting media and a glass coverslip.

Statistical analysis. Values are expressed as mean± standard error of mean (SE). Statistical differences were determined by the Tukey-Kramer test. A p-value of <0.05 was considered a statistically significant difference. Statistical analyses were performed using the Stat View 5.0 software (SAS Institute, Cary, NC).

RESULTS

The effects of a nucleic acid-rich diet on body weight, food intake and serum parameters

Final body weight (g) was significantly decreased in the DNA-Nic group compared with the C group (Table 1). The average food intake (g) was also significantly decreased in the DNA-Nic group compared with the C group (Table 1). Furthermore, the average water intake (g) was significantly decreased in the C-Nic and the DNA-Nic groups compared with the C group (Table 1).

Liver weights (g/100 g body weight) in the C-Nic and DNA-Nic group were significantly decreased compared with the C group (Table 1). However, kidney, spleen, heart, and peritesticular fat weights (g/100 g body weight) were not significantly different among the four groups (Table 1). In addition, serum glucose, triglyceride and total cholesterol levels were not significantly different among the four groups (Table 1).
A DNA-Na-rich diet suppressed elastin fiber degradation

The aortic wall thickness was not significantly different among the four groups (Fig. 1A–D, and M). Representative pictures of elastin fibers are shown in Fig. 1E–H. However, the elastin fiber destruction ratio was significantly increased in the C-Nic group compared with the C group (Fig. 1N). In addition, the elastin fiber destruction ratio was significantly suppressed in the DNA-Nic group compared with the C-Nic group (Fig. 1N). The collagen positive areas and the density of collagen fibers were measured using PSR staining (Fig. 1I–L). The collagen-positive areas were not significantly different between the C-Nic and the DNA-Nic groups (Fig. 1O).

Effect of a nucleic acid-rich diet on MMP expression

The expression of MMP-2, MMP-9 and MMP-12 in each group was examined using immunohistochemical examination (Fig. 2A–L). The area stained positive for MMP-2 in the intima-media was significantly increased in the C-Nic group compared with the C group (Fig. 2M). The MMP-2 positive areas in the intima-media were significantly decreased in the DNA-Nic group compared with the C-Nic group (Fig. 2M). The areas in the adventitia that stained positive for MMP-2 were not significantly different among the four groups (Fig. 2M).

Furthermore, the areas that stained positive for MMP-9 in the intima-media were not significantly different among the four groups (Fig. 2N). The MMP-9 positive areas in the adventitia displayed a tendency to decrease in the DNA-Nic group compared with the C-Nic group (Fig. 2N). Additionally, the MMP-12 positive areas in the vascular wall displayed a tendency to decrease in the DNA-Nic group compared with the C-Nic group (Fig. 2O).

Effect of a DNA-Na-rich diet on the expression of macrophage marker CD68, MCP-1 and MDA in the vascular wall

Immunohistochemical examination showed the expression of CD68, MCP-1 and MDA in each group (Fig. 3A–I). The CD68 positive area in the intima-media was significantly increased in the C-Nic group compared with the C group (Fig. 3M). The CD68 positive areas in the intima-media showed a tendency to decrease in the DNA-Nic group compared with the C-Nic group (Fig. 3N). Additionally, the MCP-1 positive areas in the adventitia displayed a tendency to decrease in the DNA-Nic group compared with the C-Nic group (Fig. 3O).

Furthermore, the areas that stained positive for MMP-9 in the intima-media were not significantly different among the four groups (Fig. 2N). The MMP-9 positive areas in the adventitia displayed a tendency to decrease in the DNA-Nic group compared with the C-Nic group (Fig. 2N). Additionally, the MMP-12 positive areas in the vascular wall displayed a tendency to decrease in the DNA-Nic group compared with the C-Nic group (Fig. 2O).
Dietary DNA Attenuates the Degradation of Elastin Fiber

positive for MDA in the intima-media and adventitia were not significantly different among the four groups (Fig. 3O).

DISCUSSION

In this study, we examined the effect of dietary DNA on the vascular pathology in nicotine-administered mice. The destruction of elastin fiber, which mainly exists in media, was significantly suppressed in the DNA-Nic group compared with the C-Nic group. On the other hand, the collagen positive areas, which mainly exist in adventitia, were not significantly different between the C-Nic and DNA-Nic groups. Next, we investigated the expression of MMPs associated with the degradation of fiber content in the vascular wall. The MMP-2-positive areas in the intima-media region were significantly decreased in the DNA-Nic group compared with the C-Nic group (Fig. 2M). The MMP-9 and MMP-12 positive areas in the adventitia showed decreasing trends in the DNA-Nic group compared with the C-Nic group but were not significantly different in our multiple comparison of the four experimental groups. The areas positive for CD68, MCP1, or MDA were not significantly different between the C-Nic and DNA-Nic groups. This data suggest that dietary DNA can attenuate the degradation of elastin via decreasing MMP-2 expression in vascular smooth muscle cells, which are a major cell component in the media.

Wang et al. reported that reactive oxygen species are associated with the expression of MMP-2 in vascular smooth muscle cells (8). In several AAA animal models, oxidative stress markers such as 8-isoprostane, 8-hydroxy-20-deoxyguanosine, HO-1, MDA, and inducible NOS, were reportedly increased in the diseased aortic wall (12, 22–24). In addition, increased expression of NOX and overproduction of O$_2^-$ were detected in human aneurysmal segments of aortas compared with adjacent nonaneurysmal segments (25). It has been reported that nucleotides have anti-oxidative stress capability. Cai et al. reported that alcohol-treated rats showed higher MDA and GSSG levels, with lower GSH levels and SOD activity in the liver than dextrose control rats; however, nucleotide supplementation could reverse these oxidative stress biomarkers (26). Moreover, a similar anti-oxidative activity of dietary nucleotides in aged rats has been confirmed (27). Thus, we speculated that dietary DNA could suppress oxidative stress in the vascular wall. However, the MDA-positive area, one of the oxidative markers, was not significantly changed between the C-Nic and DNA-Nic groups under our...
experimental conditions. Further studies are needed to clarify the mechanism of action of dietary DNA.

In conclusion, we have revealed the protective effect of dietary DNA on the vascular pathology of nicotine-administered mice. A nucleic acid-rich diet might be useful for people who consume nicotine via smoking, chewing tobacco, or nicotine patches.

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Supporting information
Supplemental Online Material is available on J-STAGE.

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