Sesame Extract Attenuates the Degradation of Collagen and Elastin Fibers in the Vascular Walls of Nicotine-administered Mice

Hirona Kugo¹, Chie Miyamoto¹, Ayaka Sawaragi¹, Kiyoto Hoshino¹, Yuka Hamatani¹, Shinichi Matsumura², Yuri Yoshioka², Tatsuya Moriyama¹,³, and Nobuhiro Zaima¹,³*

¹ Department of Applied Biological Chemistry, Graduate School of Agriculture, Kindai University, 3327-204 Nakamachi, Nara 631-8505, JAPAN
² INABATA KORYO, Co., Ltd., 3-5-20 Tagawa, Yodogawaku, Osaka 532-0027, JAPAN
³ Agricultural Technology and Innovation Research Institute, Kindai University, 3327-204 Nakamachi, Nara 631-8505, JAPAN

Abstract: Abdominal aortic aneurysm (AAA) is a vascular disease characterized by the weakening of the vascular walls and the progressive dilation of the abdominal aorta. Nicotine, a primary component of cigarette smoke, is associated with AAA development and rupture. Nicotine induces AAA development by weakening vascular walls. However, little is known about preventive methods using functional food factors for nicotine-induced vascular destruction. Sesamin and sesamolin are functional food factors that are fat-soluble lignans found in *Sesamum indicum* seeds. Previous reports indicated that sesamin and sesamolin have anti-oxidative and anti-inflammatory effects. In this study, we evaluated the effects of sesamin and sesamolin-rich sesame extract on the weakening of vascular walls in nicotine-administered mice. Sesame extract attenuated the degradation of collagen and elastin fibers caused by nicotine. In addition, sesame extract decreased the area positive for matrix metalloproteinase 12 (MMP-12) and oxidative stress in the vascular walls. These results suggest that sesame extract may decrease the weakening of vascular walls by suppressing the nicotine-induced degradation of collagen and elastin fibers. Sesame extract may be effective in preventing AAA development by decreasing both, MMP-12 expression and oxidative stress in vascular walls.

Key words: abdominal aortic aneurysm, smoking, nicotine, sesamin, sesamolin

1 Introduction

Abdominal aortic aneurysm (AAA) is a vascular disease characterized by chronic inflammation in the vascular wall and progressive dilation of the abdominal aorta. Previous reports indicated that the development of AAA is associated with advanced age, male sex, smoking, and hypertension. The mortality rate due to AAA rupture is very high, and therapeutic drugs to prevent the progression and rupture of AAA have not been established. The development of AAA involves various factors, including the weakening of the vascular walls due to inflammation and the degradation of fibers. Immune cells, such as monocytes and macrophages, release inflammatory cytokines, including monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α that activate matrix metalloproteinases (MMPs). Consequently, MMPs degrade the elastin and collagen fibers that play an integral role in the maintenance of vascular wall integrity and elasticity.

Cigarette smoking is a major risk factor for AAA formation. A recent study suggested that nicotine, which is a primary component of cigarette smoke, is strongly associated with AAA development and/or rupture. Wang et al. reported that nicotine infusion in Apoe-/- mice induced AAA formation. In addition, Maegdefessel et al. reported that AAA formation by nicotine is related to microRNA-21 expression. We previously reported that nicotine administration in both mice and rats caused a weakening of the vascular walls through MMPs activity, oxidative stress, and the degradation of fibers.

From the viewpoint of prevention of AAA onset or development, it is important to investigate the effect of functional food factors on the development of AAA. Previous reports using several animal models showed that dietary food components have an impact on the development or...
rupture of AAA. However, there is little information about the effect of functional food factors on the vascular damage caused by nicotine. We previously reported that eicosapentaenoic acid-rich fish oil decreases oxidative stress, MMPs activity, and the degradation of elastin fiber in the vascular walls of nicotine-administered mice. We also previously reported that dietary DNA attenuates MMP-2 expression and the degradation of elastin fibers in nicotine-administered mice. Nicotine-administered mice could be a convenient testing group for an evaluation of the effect of functional food factors on AAA-related factors. It is desirable to consume a variety of AAA-preventing functional food factors through a balanced diet. In this study, we focused on sesamin and sesamolin, which are two major fat-soluble lignans found in Sesamum indicum seeds. Sesamin and sesamolin have anti-oxidative, anti-inflammatory, and serum lipid-lowering effects. We showed the effects of dietary sesamin and sesamolin-rich sesame extract on the degradation of fibers in the vascular walls of nicotine-administered mice.

2 Experimental procedures

2.1 Sample collection
Nicotine was purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2 Animals
All animal experiments were approved by the Kindai University Animal Care and Use Committee and were performed according to the Kindai University Animal Experimentation Regulations (Approval number; KAAG-25-002). Three-week-old male C57BL/6J mice (Japan SLC, Japan), were maintained on a standard 12-hour light/dark cycle at 25 ± 1°C. After habituation for three days, the mice were divided into four groups: control diet and distilled water (C group), nicotine solution in the C group (CN group), control diet and nicotine solution (S group), and sesame extract (SN group). In addition, the mice were administered either a control diet in the C and CN groups or a sesame extract diet in the S and SN groups. All the mice were sacrificed after 16 days. The diet composition is shown in Table 1. Sesame extract contained 42.07% (w/w) sesamin and 41.23% (w/w) sesamolin.

2.3 Sample collection
The thoracic aorta was isolated, and the isolated tissue was fixed in 4% paraformaldehyde (PFA) (Nacalai Tesque, Kyoto, Japan), soaked in a sucrose solution (10%, 15%, or 20%), and embedded in optimal cutting temperature compound (Sakura Finetek Japan, Tokyo, Japan). The specimens were stored at −80°C until needed.

2.4 Histological analyses
The isolated aorta cross-sections of 5 μm thickness were prepared using a cryostat (CM1850, Leica Microsystems, Wetzlar, Germany) and mounted on glass slides. The aortic walls were stained with hematoxylin-eosin (HE), Elastica van Gieson (EVG), Picrosirius red (PSR), and immunohistochemical stainings. The quantitative analyses of the histologically stained sections were performed using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA), and the destruction rate of the wavy configuration of the elastic lamina was calculated as previously described.

2.5 Immunohistochemical staining
Immunohistochemical staining was performed as previously described. The histological results from the aortic wall were assessed after staining using the following antibodies: rabbit anti-MMP-2 (1:50; Thermo Scientific, San Jose, CA, USA), goat anti-MMP-9 (1:50; Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-MMP-12 (1:100; Bioss Antibodies, Woburn, MA, USA), mouse anti-malondialdehyde (MDA) (1:100; Abcam, Tokyo, Japan), rabbit anti-monocyte chemotactic protein-1 (MCP-1) (1:50; Bioss Antibodies, Woburn, MA, USA), and rabbit anti-CD68 (1:50; Bioss Antibodies, Woburn, MA, USA).

2.6 Statistical analyses
The values were expressed as the mean ± standard error of mean (S.E.M). Statistical differences were determined by the Tukey-Kramer test, and a p-value < 0.05 was considered statistically significant. Statistical analyses were performed using Stat View 5.0 software (SAS Institute, Cary, USA).

Table 1: Diet composition.

<table>
<thead>
<tr>
<th></th>
<th>Control diet (g)</th>
<th>Sesame extract diet (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline chloride</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>AIN-93 vitamin mix</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>AIN-93G mineral mix</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>52.95</td>
<td>52.95</td>
</tr>
<tr>
<td>Olive oil</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Sesame extract</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>Total (g)</td>
<td>100.0</td>
<td>101.0</td>
</tr>
</tbody>
</table>
3 Results

3.1 Effects of dietary sesame extract on body weight and food intake

Final body weight (g) was significantly decreased in the S and SN groups compared to the C group (Fig. 1a). The average food intake (g/day) was not significantly different between the four groups (Fig. 1b).

3.2 Suppressive effects of dietary sesame extract on collagen and elastin fibers degradation

The thickness of the aortic wall was not significantly different between the four groups (Fig. 2a-e). The collagen-positive areas were significantly decreased in the CN group compared to the C and S groups (Fig. 2f-j), and they were significantly increased in the SN group compared to the CN group (Fig. 2f-j). The elastin fiber destruction area was significantly increased in the CN group compared to that in the C and S groups (Fig. 2k-o), and it was significantly decreased in the SN group compared to that in the CN group (Fig. 2k-o).

3.3 Effects of dietary sesame extract on MMPs expressions and oxidative stress

The areas positive for MMP-2 in the intima-media and adventitia of the aortic wall were significantly increased in the CN group compared to the C group (Fig. 3a-e). The areas positive for MMP-2 in the intima-media of the aortic wall tended to decrease in the SN group compared to the CN group (Fig. 3a-e). The areas positive for MMP-2 in the intima-media and adventitia of the aortic wall were significantly increased in the SN group compared to the CN group (Fig. 3a-e). The areas positive for MMP-2 in the intima-media of the aortic wall were significantly increased in the SN group compared to the CN group (Fig. 3a-e).

Fig. 1 Body weight change and food intake. (a) Body weight change of mice and (b) food intake for the four experimental groups. Data are expressed as the mean ± S.E.M. C group (n = 6), S group (n = 6), CN group (n = 6), and SN group (n = 7). *p < 0.05 in the S and SN groups versus the C group.

Fig. 2 Hematoxylin-eosin (HE), Elastica van Gieson (EVG), and Picrosirius red (PSR) staining. Representative images of HE staining (a-d) and quantification of vascular wall thickness in the four experimental groups (e). Representative images of PSR staining (f-i) and quantification of collagen positive area in the four experimental groups (j). Representative images of EVG staining (k-n) and quantification of the destruction rate of the elastic lamina waveform (o). Scale bar = 50 μm. Data are expressed as the mean ± S.E.M. C group (n = 6), S group (n = 6), CN group (n = 6), and SN group (n = 7). Values with different letters are significantly different (p < 0.05).

Fig. 3  Immunohistochemical staining for matrix metalloproteinase (MMP)-2, MMP-9, and MMP-12. Representative images of immunostaining for MMP-2 (a-d) and quantification of MMP-2 positive areas of the vascular wall (e). Representative images of immunostaining for MMP-9 (f-i) and quantification of MMP-9 positive areas of the vascular wall (j). Representative images of immunostaining for MMP-12 (k-n) and quantification of MMP-12 positive areas of the vascular wall (o). Scale bar = 50 μm. Data are expressed as the mean ± S.E.M. C group (n = 6), S group (n = 6), CN group (n = 6), and SN group (n = 7). Values with different letters are significantly different (p < 0.05).

Fig. 4  Immunohistochemical staining for monocyte chemotactic protein (MCP)-1, CD68⁺ monocyte/macrophages and malondialdehyde (MDA). Representative images of immunostaining for MCP-1 (a-d) and quantification of MCP-1 positive areas of the vascular wall (e). Representative images of immunostaining for CD68⁺ monocyte/macrophages (f-i) and quantification of CD68⁺ monocyte/macrophages positive areas of the vascular wall (j). Representative images of immunostaining for MDA (k-n) and quantification of MDA positive areas of the vascular wall (o). Scale bar = 50 μm. Data are expressed as the mean ± S.E.M. C group (n = 6), S group (n = 6), CN group (n = 6), and SN group (n = 7). Values with different letters are significantly different (p < 0.05).

CN group (Fig. 3a-e). The areas positive for MMP-9 in the aortic wall were not significantly different between the four groups (Fig. 3f-j). The areas positive for MMP-12 in the intima-media of the aortic wall were significantly increased.
in the CN group compared to the C and S groups (Fig. 3k-o), and they were significantly decreased in the SN group compared to the CN group (Fig. 3k-o).

The areas positive for MCP-1 and CD68 in the aortic wall were not significantly different between the four groups (Fig. 4a-j). The areas positive for MDA, an oxidative stress marker, in the intima-media of the aortic wall were significantly increased in the CN group compared to those in the C and S groups (Fig. 4k-o), and they were significantly decreased in the SN group compared to those in the CN group (Fig. 4k-o).

4 Discussion

In this study, we evaluated the effects of dietary sesame extract on vascular fiber degradation in nicotine-administered mice. A histological analyses showed that the dietary sesame extract attenuated the degradation of collagen and elastin fibers in the nicotine-administered mice (Fig. 2j, o). The dietary sesame extract also decreased the areas positive for MMP-12 and oxidative stress in the vascular walls of the nicotine-administered mice (Fig. 3o and Fig. 4o). These results suggest that sesame extract may decrease the weakening of vascular walls by suppressing the degradation of the collagen and elastin fibers caused by nicotine.

In this study, the administration of nicotine facilitated MMPs expressions, oxidative stress, and the degradation of collagen and elastin fibers. Jacob-Ferreira et al. demonstrated that nicotine increased MMP activity in the aortic wall. Wang et al. reported that a nicotine-induced increase in reactive oxygen species in vascular smooth muscle cells facilitated MMP-2 expression and AAA formation. The phosphorylation of activator protein 2e caused by the activation of adenosine monophosphate-activated protein kinase α by the activation of adenosine monophosphate-activated protein kinase (MAPK) and protein kinase C, and these molecules then activate AP-1 and NFκB. We suggest that the expression of MMP-12 in this study may be induced by nicotine-mediated activation of the p38 MAPK and NFκB pathways. Dietary sesame extract significantly decreased the areas positive for MMP-12 in the nicotine-administered mice (Fig. 3o). Previous reports showed that sesamin might inhibit the activation of p38 MAPK and NFκB. Therefore, the suppression of MMP-12 expression by dietary sesame extract may be mediated by the p38 MAPK and NFκB pathways.

5 Conclusion

Our results suggest that dietary sesame extract may suppress the degradation of collagen and elastin fibers in nicotine-administered mice by decreasing MMP-12 expression and oxidative stress. We speculate that the anti-oxidative and anti-inflammatory effects of sesamin and sesamolin in sesame extract may decrease the nicotine-caused oxidative stress and inflammation in the vascular walls. Food that contains sesamin and sesamolin may be effective in preventing the development of AAA by inhibiting the effects of nicotine. Avoiding nicotine intake by smoking is one of the most important methods in AAA treatment, but it is difficult to quit smoking immediately due to dependency on nicotine. The function of sesame extract on nicotine may be used to provide the novel preventing method for those who cannot quit smoking.

Acknowledgements

This work was supported by Grant-in-Aid for Scientific Research (B) (17H03822) (to N.Z.) from the Japan Society for the Promotion of Science.

Conflicts of Interest

The authors declare that there is no conflict of interest.

References

3) Lindsay, M.E.; Dietz, H.C. Lessons on the pathogenesis of aneurysm from heritable conditions. Nature 473, 83


26) Curci, J.A.; Liao, S.; Huffman, M.D.; Shapiro, S.D.; Thompson, R.W. Expression and localization of macro-

84
Effect of Sesame Extract on Aortic Wall


