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Varenicline aggravates plaque formation through α7 nicotinic acetylcholine receptors in ApoE KO mice

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ABSTRACT

Varenicline is one of the most widely used drugs for smoking cessation. However, whether an adverse effect of varenicline is associated with the risk of serious cardiovascular events remains controversial. In this study, we determined if varenicline increases the risk of cardiovascular events using apolipoprotein E knockout (ApoE KO) mice. ApoE KO mice (8 weeks old) were injected with varenicline 0.5 mg kg^{-1} day^{-1} for 3 weeks. Varenicline aggravated atherosclerotic plaque formation in whole aorta from ApoE KO mice compared with vehicle. Methyllycaconitine, an α7 nicotinic acetylcholine receptor (nAChR) antagonist, inhibited varenicline-induced aggravated plaque formation. Our findings show that varenicline progresses atherosclerotic plaque formation through α7 nAChR, and thereby increases the risk of cardiovascular events.

1. Introduction

Smoking is an important causative and/or risk factor for the induction and development of cardiovascular disease, ischemic heart disease, chronic obstructive pulmonary disease, and cancer [1,2]. Among tobacco users, cardiovascular disease occurs with high morbidity, and is a leading cause of death. Smoking cessation is well known to produce long-term cardiovascular benefits and is strongly recommended for patients with various diseases [3].

Varenicline has recently been introduced as an aid to smoking cessation. It is a partial agonist of α4β2 nicotinic acetylcholine receptors (nAChRs), and a full agonist of α7 nAChR [4,5]. Varenicline is more effective than nicotine replacement therapy and frequently used clinically for smoking cessation [6–8]. However, varenicline shows adverse effects, most commonly headache, nausea, abnormal dreams, and insomnia [9,10]. In addition to these central adverse reactions, increased risk of cardiovascular events has been observed in patients taking varenicline [10,11], although there are also reports showing no significant increase in severe cardiovascular adverse events [12–14]. Thus, involvement of varenicline and increased cardiovascular risk remains controversial. To determine if varenicline treatment is associated with cardiovascular events, we examined the effect of long-term varenicline treatment on atherosclerotic plaque formation in apolipoprotein E knockout (ApoE KO) mice.

2. Materials and methods

C57BL/6J ApoE KO mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and housed under standard conditions for humidity, room temperature, and dark-light cycles. Mice were given free access to food and water throughout the study. The study protocol was approved by the Laboratory Animal Care and Use Committee of Fukuoka University.

2.1. Drugs

Varenicline tartrate was purchased from Toronto Research Chemicals Inc. (Toronto, ON, Canada) and dissolved in saline (Otsuka Pharmaceutical Co., Tokyo, Japan).

2.2. Experiment 1

ApoE KO mice (8 weeks old) were fed a high-fat diet (1.25% cholesterol, 15% cacao butter, and 0.5% sodium cholate, F2HFD1; Oriental Yeast Co., Tokyo, Japan). Mice were randomized into varenicline-treated and non-treated groups, and subcutaneously injected with either saline (vehicle, $n = 8$) or 0.05/0.5 mg kg^{-1}

Abbreviations: nAChRs, nicotinic acetylcholine receptors; ApoE KO mice, apolipoprotein E knockout mice; MLA, methyllycaconitine.

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day^{-1} varenicline (n = 9/8, respectively) for a 3-week period starting from 8 weeks old. At 11 weeks of age, mice were killed and perfused with ice-cold phosphate-buffered saline. The heart and aorta were excised and analyzed histologically.

2.3. Experiment 2

ApoE KO mice (8 weeks old) were fed a high-fat diet, and randomized into the following three groups: (1) vehicle group treated with saline (n = 10), (2) varenicline group treated with 0.5 mg kg^{-1} day^{-1} varenicline (n = 10), and (3) methyllycaconitine (MLA) plus varenicline group injected with 5 mg kg^{-1} day^{-1} MLA (an α7 nAChR antagonist) into the abdominal cavity and 0.5 mg kg^{-1} day^{-1} varenicline (n = 9) for 3 weeks. At 11 weeks of age, mice were euthanized and the *en face* plaque area examined.

2.4. En-face plaque area

To quantify the extent of atherosclerotic lesions, immediately after mice were killed, the whole aortic length was excised for quantification of the *en face* plaque area, as previously described [15–17]. Briefly, after carefully removing fat and adventitial tissue, the aortic arch and thoracic to abdominal aorta were opened longitudinally, pinned on a black wax surface, and stained with oil red O (Sigma, St. Louis, MO, USA). *En face* images were obtained using a stereomicroscope and analyzed with the public domain software, Image J (NIH Image, Bethesda, MD, USA). Percentage of the luminal surface plaque area stained by oil red O was determined.

2.5. Histological analysis and oil red O staining

Lipid accumulation in atherosclerotic plaques at the aortic root in the heart was analyzed. The aortic root was embedded in O.C.T. Compound (Sakura FineTech, Tokyo, Japan). Serial cryostat sections (6 μm thick) were prepared as described previously [15–17]. Briefly, atherosclerotic plaques were investigated in five separate sets of sections, with each set separated by 60 μm. Oil red O staining was performed to examine lipids. The oil red O-positive area, a marker of lipid accumulation, was analyzed using Image J. Average values for the five sections in each animal were used for analysis.

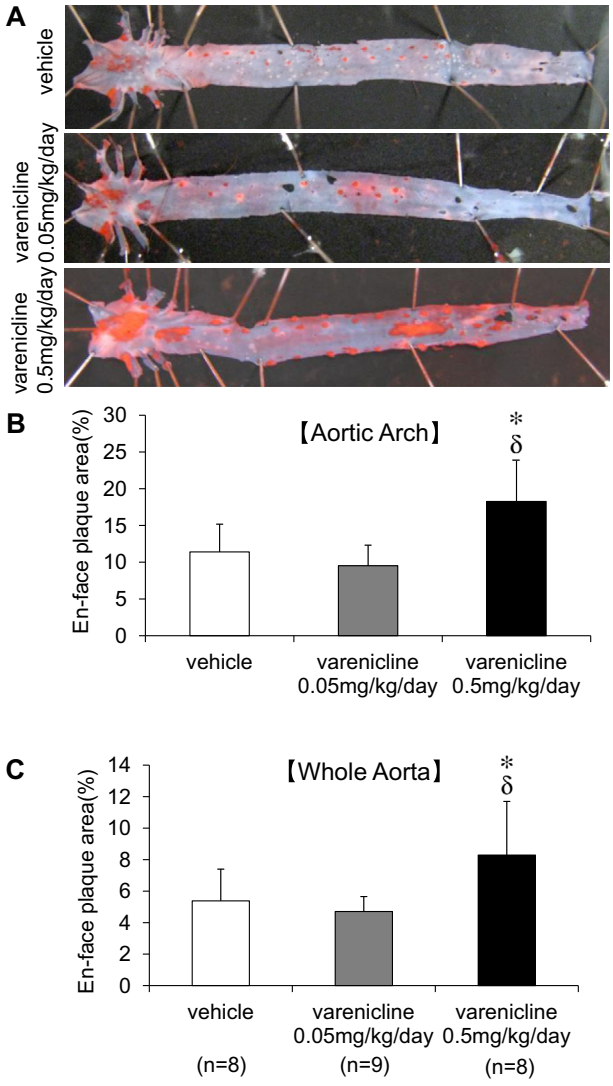

Fig. 1. Atherosclerotic plaques in the aorta of varenicline-treated ApoE KO mice. (A) Representative *en face* photographs of the aorta showing oil red O-stained atherosclerotic plaques. Quantitative measurement of *en face* plaque area (%) in the aortic arch (B) and whole aorta (C). Each bar indicates mean ± S.D. $^{*}P < 0.05$ vs vehicle group, $^{\delta}P < 0.05$ vs 0.05 mg kg^{-1} day^{-1} varenicline treatment group.

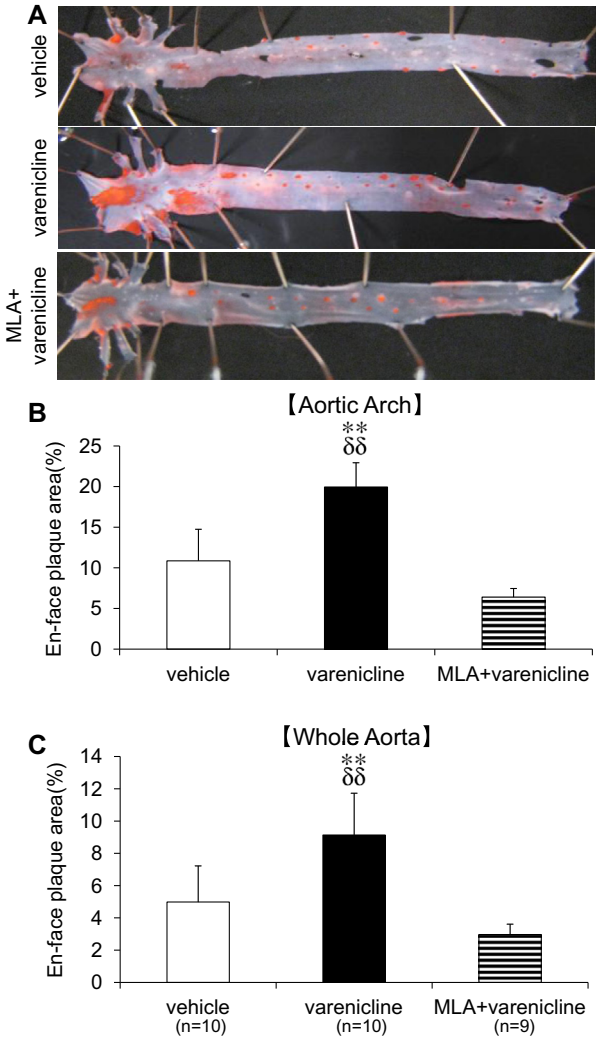

Fig. 2. Atherosclerotic plaques in the aorta of varenicline- and varenicline plus MLA-treated ApoE KO mice. (A) Representative *en face* photographs of the aorta showing oil red O-stained atherosclerotic plaques from vehicle, varenicline, and varenicline + MLA-treated ApoE KO mice. Pooled data showing the effect of varenicline and MLA treatment on oil red O-stained positive areas in the aortic arch (B) and whole aorta (C). Each bar indicates mean ± S.D. $^{**}P < 0.01$ vs vehicle group, $^{\delta\delta}P < 0.01$ vs varenicline treatment group.

2.6. Measurement of total plasma cholesterol levels

At 11 weeks of age, blood was collected to measure plasma total cholesterol levels. Plasma total cholesterol levels were measured using WAKO Cholesterol E Assay kit (Wako Chemical Co., Osaka, Japan).

2.7. Statistical analysis

Histological quantitative analyses were performed by a single observer blinded to the experimental protocol. Data are expressed as mean ± standard deviation. Bonferroni analysis was used for comparison between the three groups. *P* values <0.05 were considered statistically significant.

3. Results

3.1. Experiment 1

We determined if varenicline causes progression of plaque formation in ApoE KO mice. Varenicline at a dose of 0.5 (but not 0.05) mg kg^{-1} day^{-1} for 3 weeks significantly progressed plaque formation in the aortic arch and whole aorta, in ApoE KO mice compared with vehicle and low-dose (0.05 mg kg^{-1} day^{-1}) varenicline groups (Figs. 1 and 2). Plaques in the aortic arch and whole aorta formed by varenicline treatment (0.5 mg kg^{-1} day^{-1} for 3 weeks) were 1.6- and 1.5-fold increased, respectively, compared with vehicle. This higher dose of varenicline showing significant and marked aggravation in plaque formation was employed in the following experiment.

3.2. Experiment 2

To determine the mechanism by which varenicline aggravates atherosclerotic plaque formation, we examined the effect of MLA, an α7 nAChR antagonist, on varenicline-induced aggravation of atherosclerotic plaque formation in ApoE KO mice. With combined varenicline and MLA treatment, varenicline-induced aggravation of atherosclerotic plaque formation in the aortic arch and whole aorta of ApoE KO mice were not observed (Fig. 2). Moreover, atherosclerotic plaque formation was markedly increased by 178% in the aortic root of the varenicline group (Fig. 3). MLA also inhibited varenicline-induced aggravated plaque formation in the aortic root (Fig. 3). There were no significant differences in body weight and total plasma cholesterol levels between vehicle, varenicline, and MLA plus varenicline groups (Table 1).

Table 1
There are no significant differences in body weight or total cholesterol between groups. Data are expressed as mean ± S.D. *N* = 10 (vehicle), 10 (varenicline), and 9 (MLA plus varenicline).

	Vehicle	Varenicline	MLA + varenicline
Body Weight, g	18.4 ± 2.6	18.4 ± 1.9	18.7 ± 2.6
Total Cholesterol, mg/dl	1952 ± 306	1920 ± 374	1997 ± 454

4. Discussion

Patients with cardiovascular disease, even though receiving drug therapy, are at high risk for the occurrence of cardiovascular events. To decrease this risk, patients are strongly recommended to stop smoking [1]. Varenicline is one of the most effective drugs for smoking cessation [6–8]; however, when compared with a placebo, an increased risk of cardiovascular events has been reported in patients taking varenicline [10,11]. Therefore, in this study, we determined if varenicline causes progression of plaque formation in ApoE KO mice. Long-term varenicline treatment at a dose of 0.5 mg kg^{-1} day^{-1} progressed plaque formation in the aortic arch and whole aorta of ApoE KO mice (Fig. 1), suggesting that varenicline may increase the risk for occurrence of cardiovascular events.

Varenicline is an α4β2 nAChR partial agonist and an α7 nAChR full agonist [5]. Varenicline efficacy in smoking cessation is due to

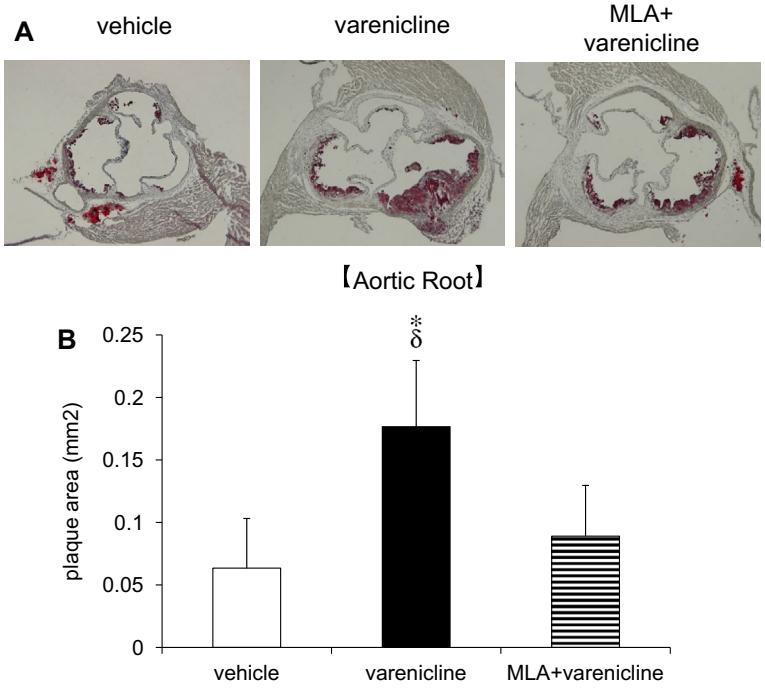

Fig. 3. Atherosclerotic plaques in the aorta of varenicline- and varenicline plus MLA-treated ApoE KO mice. (A) Representative cross-sections of oil red O-stained plaques in the aortic root. (B) Pooled data of plaque area in the aortic root. Each bar indicates mean ± S.D. $^{*}P < 0.05$ vs vehicle group, $^{\delta}P < 0.05$ vs varenicline treatment group.

its partial agonistic activity at neuronal α4β2 nAChRs in the brain mesolimbic dopaminergic system. Varenicline prevents nicotine from binding to and moderately stimulates these receptors, leading to inhibition of nicotine reinforcement during smoking and reduced craving during smoking abstinence. Nevertheless, varenicline efficacy in α7 nAChR activation is 8-fold less potent than at α4β2 nAChRs [5]. Considering that in the clinic long-term varenicline treatment is ongoing for several months, it is highly likely that a significant effect of varenicline occurs because of activation of α7 nAChR. The α7 nAChR is expressed on cells associated with atherosclerotic plaque formation, including human vascular smooth muscle cells, aortic endothelial cells, platelets, macrophages, and T and B lymphocytes. Among these human cell types, the α4 subunit is expressed on vascular smooth muscle cells and B lymphocytes, while the β2 subunit is expressed on aortic endothelial cells and T lymphocytes [18–21]. Therefore, varenicline appears to aggravate plaque formation by acting on α7 rather than α4β2 nAChRs. This is likely associated with varenicline-induced cardiovascular events, although further experiments are required to clarify the target cell of varenicline-induced aggravation of plaque formation.

To determine if varenicline aggravates atherosclerosis formation via α7 nAChR, varenicline was administered to ApoE KO mice in combination with MLA, an α7 nAChR antagonist. As shown in Figs. 2 and 3, MLA inhibited varenicline-aggravated plaque formation in the aortic arch, whole aorta, and aortic root of ApoE KO mice. These results strongly support the notion that varenicline aggravates plaque formation through α7 nAChR, and thereby causes adverse cardiovascular reactions. In addition, there are reports suggesting that the α7 nAChR play a crucial role in the mediation of nicotine-induced dendritic cell maturation, platelet activation, and E-selectin expression in aortic endothelial cells [21–23]; all phenomena involved in the progression of atherosclerotic plaque formation. Furthermore, one case report found that varenicline has a prothrombotic effect via brainstem α7 nAChR [24]. This is similar to the effects of nicotine, because acute nicotine administration is capable of inducing thrombosis. These results support our present findings that demonstrate α7 nAChR involvement in adverse cardiovascular reactions of varenicline.

In conclusion, varenicline aggravates plaque formation by stimulating α7 nAChR, and consequently may increase the risk for cardiovascular events. The possibility that varenicline is involved in adverse cardiovascular effects through α7 nAChR must be considered.

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