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SHORT COMMUNICATION

Preventive effect of varenicline on impairment of endothelial function in cerebral vessels induced by acute smoking in rats

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Abstract Preoperative smoking cessation is important for recovery from surgery without complications. Available evidence suggests nicotine replacement therapy could be safe and effective in the perioperative period. On the other hand, the newly developed selective nicotinic acetylcholine (ACh) receptor partial agonist, varenicline tartrate, is also effective as an aid for smoking cessation and helps people to stop smoking. During the transitional phase between the decision to stop smoking and actual smoking cessation, patients could use varenicline before undertaking smoking cessation. We have previously reported that acute cigarette smoking can cause impairment of endothelium-dependent vasodilation in cerebral vessels; thus, the use of varenicline before surgery in a patient who is still a smoker may not be safe with regard to endothelial function. Therefore, to assess the safety of varenicline in terms of endothelial function, we evaluated its effect on the acute smoking-induced impairment of endothelium-dependent cerebral vasodilation. In anesthetized Sprague–Dawley rats, we applied ACh topically to pial vessels; then, after administering intravenous varenicline or saline injection, we reexamined the ACh-induced vasodilator response both before and after smoking. Under control conditions, cerebral pial arterioles were dose-relatedly dilated by ACh. After smoking, 10^{-5} M ACh constricted the arterioles following saline pretreatment (diameter -7.6 ± 1.8 %, $n = 6$), but induced dilation following varenicline pretreatment (diameter $+15.3 \pm 3.3$ %, $n = 6$). Thus, varenicline may prevent the smoking-induced impairment of endothelium-dependent vasodilation in cerebral pial arterioles.

Keywords Smoking · Varenicline · Cerebral circulation · Endothelial function

Previous studies have shown that smoking can cause functional alterations in the endothelium, leading to impaired endothelium-dependent dilation in cerebral vessels [1]. We previously reported that, in rats, acute cigarette smoking, but not nicotine itself, impaired endothelium-dependent vasodilation in cerebral vessels [2–5]. Stopping smoking is important for cardiovascular patients not only for primary but also for secondary prevention [6]. Although nicotine has cardiovascular effects, the available evidence suggests that nicotine-replacement therapy may be safe and effective in the perioperative period even in patients with cardiovascular disease [7]. Varenicline, a newly developed selective partial agonist of the nicotinic acetylcholine (ACh) receptor, is an effective aid for the cessation of smoking [8]. Varenicline, which does not contain nicotine, works in two ways. It targets nicotine receptors within the brain, attaches to them, and activates them, causing a reduced release of dopamine (compared with nicotine), and it also prevents nicotine reaching the receptors [9]. Although patients should stop smoking completely by day 8 of varenicline treatment, they may still be smoking

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during the first week of the treatment. There is little information about the effects varenicline on cerebral vessels, including endothelial function. Therefore, giving varenicline for a week or so during the transitional phase before smoking cessation may not be safe for patients with regard to endothelial function.

We aimed to evaluate the effect of prior varenicline on the smoking-induced impairment of endothelium-dependent cerebral vasodilation in rats in vivo. We studied 22 male Sprague–Dawley rats weighing 300–350 g. The experimental protocols were approved by the Institutional Committee for Animal Care of Gifu University Graduate School of Medicine. Each rat was anesthetized (pentobarbital sodium, 50 mg/kg body weight, i.p.), then mechanically ventilated (60/min) through a tracheostomy tube by means of a ventilator (KN-56; Natsume Seisakusho, Tokyo, Japan) using room air supplemented with oxygen. At the beginning of each experiment, tidal volume was adjusted to maintain $PaCO_2$ at between 35 and 40 mmHg, using a pressure-limited system. Supplementary pentobarbital was administered intravenously by continuous infusion at 4 mg/kg/h. One femoral artery was cannulated, both for the continuous measurement of arterial blood pressure and heart rate (HR) and to provide blood samples for the determination of arterial blood gas tension, pH, glucose, and serum electrolytes. A femoral vein was cannulated for the administration of fluid and drugs. Rectal temperature was maintained at between 37 and 38 °C by means of a heating pad. The methodology used here has been detailed previously [2–5]. In brief, a closed cranial window made of a polypropylene ring with a fitted glass coverslip was used for observation of the pial microcirculation. Four catheters were inserted into the ring: one was attached to a reservoir bottle containing artificial cerebrospinal fluid (aCSF) to maintain constant intracranial pressure, the second was for the continuous monitoring of intracranial pressure, the third was for the administration of experimental drugs and aCSF, and the fourth was for draining the fluid. The temperature within the window, which was monitored using a thermistor (Model 6510; Mallinckrodt Medical, St. Louis, MO, USA), was maintained at 37.0–38.0 °C. In each rat, the pial views obtained in these experiments were stored on videotape (with a time record) for later playback and analysis. In each rat, the diameters of three cerebral pial arterioles (baseline diameters 30–100 μm) were measured using a videomicrometer (Olympus Flovel videomicrometer, Model VM-20; Flovel, Tokyo, Japan) on a television monitor receiving signals from a microscope (Model SHZ-10; Olympus, Tokyo, Japan). We selected the vessels from which data were to be collected at the very beginning of the experiment (before drug administration) to eliminate bias as far as possible. Values representing percentage changes in pial vessel diameter were used in the statistical analysis. Cigarette smoking was performed as described in previous publications (inhaling 60 puffs per min of mainstream cigarette smoke for 1 min (1 mg-nicotine cigarette; Marlboro, Philip Morris) [2] by way of the ventilator). The test responses were the vasodilator responses to the topical continuous infusion (0.2 mL/min for 5 min) of an endothelium-dependent vasodilator (ACh at 10^{-6} and 10^{-5} M). Pial arteriolar diameters were measured after 4-min continuous infusion of ACh. We reexamined the vasodilator response to topical ACh 10^{-5} M 30 min after the intravenous injection of varenicline tartrate (Pfizer, New York, NY, USA) (1 mg/kg, 1 mg/ml in saline; $n = 6$) or saline ($n = 6$), and again 1 h after cigarette smoking. In another experiment, we measured the serum 8-isoprostane level (by an enzyme-linked immunosorbent assay [ELISA]) as a marker of oxidative stress before and 1 h after 1-min cigarette smoking in rats injected with saline ($n = 5$) or varenicline tartrate ($n = 5$) as above. Changes in all variables (dose-dependent effects of ACh, and the effects of smoking) were examined via a one-way analysis of variance (ANOVA) for repeated measurements, with a paired *t*-test (with a Bonferroni correction) being used for post-hoc comparisons. The group effects of pretreatment with saline or varenicline were compared by an unpaired *t*-test. Significance was set at $P < 0.05$. All values are presented as means ± SD.

We found that cerebral pial arterioles were dilated dose-relatedly by topical ACh (10^{-6} and 10^{-5} M) in both the varenicline group and the control group (Fig. 1). Neither saline nor varenicline altered the response to topical 10^{-5} M ACh. One hour after a 1-min inhalation of

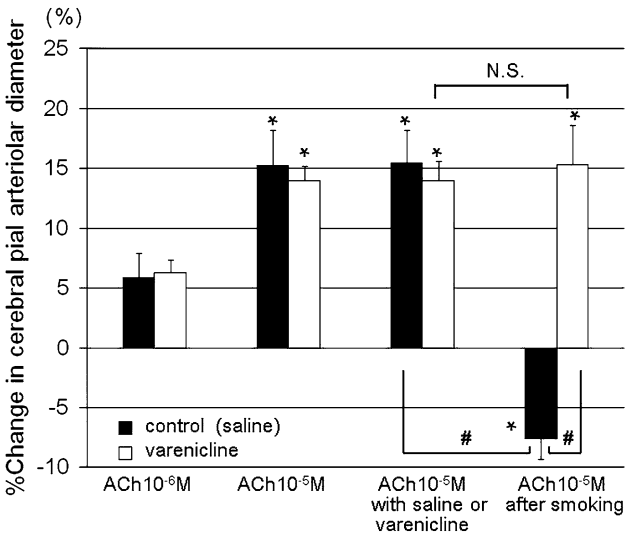

Fig. 1 Effects of topically applied acetylcholine (*ACh*) on the diameter of rat pial arterioles before and after saline or varenicline injection, and after subsequent cigarette smoking. *$P < 0.05$ versus 10^{-6} M ACh; # and *N.S.*, $P < 0.05$ or not significant, respectively, for differences between the indicated values

mainstream smoke, 10^{-5} M ACh constricted cerebral pial arterioles (-7.6 ± 1.8 %) in the control group, indicating that the vasodilator response to topical ACh was impaired after smoking. In contrast, in the varenicline group, 1 h after smoking, 10^{-5} M ACh dilated cerebral pial arterioles by 15.3 ± 3.3 % ($p < 0.05$ vs. control group). The post-smoking levels of 8-isoprostane were not different between the varenicline and control groups (data not shown). Plasma glucose was increased by smoking in both groups (Table 1). However, the other parameters tested showed no significant changes throughout the experiment in either group (Table 1). There were no differences between the groups in any of these parameters.

The published experimental trials suggest that nicotine-replacement therapy (NRT) does not adversely affect, or may indeed improve, many of the factors contributing to cardiovascular risk [10]. NRT has been proven to be safe in patients with coronary artery disease, even if they continue to smoke [11]. Varenicline, which has recently been approved for the treatment of nicotine dependence, is a partial agonist at the alpha4beta2 nicotinic acetylcholine receptor. As a partial agonist, it may serve both to treat the symptoms of nicotine withdrawal and to block the pleasurable effects of nicotine if cigarettes are smoked [9]. The sympathomimetic cardiovascular effects of nicotine, which are mediated primarily by binding to alpha3beta4 nicotinic acetylcholine receptors [12], include increases in heart rate, myocardial contractility, and blood pressure. Because of its relative selectivity for alpha4beta2 nicotinic acetylcholine receptors, varenicline is predicted to have no cardiovascular effects. However, there is little information about the effects of varenicline on cerebral vessels, including endothelial function. In the present study, varenicline itself did not change the response to ACh, but it prevented the smoking-induced impairment of endothelium-dependent vasodilation in cerebral pial arterioles. The mechanisms underlying this protection of endothelial function was not clarified by our measurements of serum 8-isoprostane as a marker of oxidative stress, and so further investigation will be needed. Nevertheless, if our results can be extrapolated to humans,

Table 1 Changes in hemodynamic and physiological parameters during experiments

	HR (beats/min)	MABP (mmHg)	pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Na (mEq/L)	K (mEq/L)	Glucose (mg/dl)	WT (°C)
Control group ($n = 6$)									
Before smoking									
Before ACh10^{-6} M	355 ± 79	127 ± 10	7.44 ± 0.01	36.7 ± 2.5	232 ± 37	145 ± 3	3.5 ± 0.2	117 ± 12	37.3 ± 0.3
After	351 ± 72	127 ± 15							
Before ACh10^{-5}M	327 ± 52	124 ± 19	7.44 ± 0.03	35.9 ± 1.4	239 ± 43	145 ± 2	3.6 ± 0.2	120 ± 12	37.2 ± 0.4
After	327 ± 59	127 ± 18							
After saline i.v.									
Before ACh10^{-5} M	334 ± 68	127 ± 17	7.43 ± 0.02	36.1 ± 1.1	235 ± 36	145 ± 2	3.7 ± 0.1	122 ± 12	37.2 ± 0.4
After	336 ± 65	126 ± 18							
After smoking									
Before ACh10^{-5} M	346 ± 62	127 ± 10	7.44 ± 0.04	37.1 ± 2.6	243 ± 50	144 ± 2	3.6 ± 0.2	136 ± 11*	37.2 ± 0.5
After	344 ± 53	124 ± 11							
Varenicline group ($n = 6$)									
Before smoking									
Before ACh10^{-6}M	388 ± 22	119 ± 11	7.43 ± 0.03	35.6 ± 1.9	229 ± 47	142 ± 2	3.5 ± 0.3	125 ± 9	37.3 ± 0.3
After	388 ± 22	119 ± 11							
Before ACh10^{-5} M	388 ± 22	124 ± 9	7.43 ± 0.03	35.3 ± 1.0	224 ± 44	142 ± 1	3.8 ± 0.3	131 ± 11	37.2 ± 0.4
After	392 ± 22	121 ± 13							
After varenicline i.v.									
Before ACh10^{-5} M	405 ± 30	123 ± 8	7.42 ± 0.04	35.1 ± 0.5	232 ± 47	143 ± 1	3.6 ± 0.5	137 ± q17	37.3 ± 0.2
After	407 ± 31	121 ± 8							
After smoking									
Before ACh10^{-5} M	422 ± 31	121 ± 10	7.41 ± 0.03	36.9 ± 1.9	239 ± 59	142 ± 2	3.4 ± 0.3	147 ± 11*	37.4 ± 0.4
After	418 ± 37	122 ± 13							

Values are means ± SD

WT intra-window temperature, *HR* heart rate, *MABP* mean arterial blood pressure, *ACh* acetylcholine

* $p < 0.05$ versus before ACh 10^{-6} M in "before smoking" category

they suggest that varenicline could be used safely, in terms of endothelial function, during the week or so before patients are attempting to quit smoking in the perioperative period.

In conclusion, varenicline was shown to restore the smoking-induced impairment of endothelium-dependent vasodilation in cerebral pial arterioles. Accordingly such an effect could be favorable, in terms of endothelial function in cerebral vessels, for the use of varenicline in the perioperative period.

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