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A lipoprotein lipase activator, NO-1886, improves endothelium-dependent relaxation of rat aorta associated with aging

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Abstract

Endothelial function is closely related to development of atherosclerosis and is impaired with aging. The novel compound NO-1886, 4-diethoxyphosphorylmethyl-*N*-(4-bromo-2-cyanophenyl)benzamide, is a lipoprotein lipase activator and its long term administration protects against the development of experimental atherosclerosis in animals. The aim of this study was to ascertain whether NO-1886 ameliorates the impaired endothelium-dependent relaxation of rat aorta associated with aging. NO-1886 (50 mg/kg p.o.) was administered to 7-month old rats for 3 months. Plasma lipid, glucose and insulin levels in old control rats (10 months of age) were significantly higher than those of young rats (2 months of age). NO-1886 decreased plasma triglyceride levels (old rats, 233 ± 10 mg/dl; old rats + NO-1886, 172 ± 16 mg/dl, $P < 0.01$) and increased plasma high density lipoprotein (HDL) cholesterol level (old rats, 72 ± 6 mg/dl; old rats + NO-1886, 142 ± 6 mg/dl, $P < 0.001$) in old rats, but had no effects on plasma glucose or insulin. The endothelium-dependent relaxation of the thoracic aorta caused by histamine was significantly impaired in old rats (% relaxation at $10^{-5.5}$ M histamine: young rats $25.4 \pm 3.1\%$; old rats $14.1 \pm 1.9\%$, $P < 0.01$), an effect completely prevented by NO-1886 (old rats + NO-1886; $22.8 \pm 2.8\%$, $P < 0.05$ vs. old rats). In contrast, NO-1886 showed no effect on the endothelium-independent relaxation by sodium nitroprusside. These results indicate that NO-1886 improves impaired endothelium-dependent relaxation of rat aorta associated with aging, possibly by correcting lipid metabolism.

Keywords: NO-1886; Lipoprotein lipase; Endothelium-dependent relaxation; High density lipoprotein cholesterol; (Rat, old)

1. Introduction

Impaired endothelium-dependent relaxation of arteries has been reported in a number of species (Andrews et al., 1987; Bossaller et al., 1987; Freiman et al., 1986; Jayakody et al., 1987; Ludmer et al., 1986; Verbeuren et al., 1986; Yamamoto et al., 1987): It is closely related to development of atherosclerosis and exists before the development of intimal lesions in hypercholesterolaemic animals (Shimokawa and Vanhoutte, 1989). Aging is a risk factor for cardiovascular disease (Lakatta and Yin, 1982). In the arteries of rats, endothelium-dependent relaxation decreases with age (Moritoki et al., 1986, 1988; Mayhan et al., 1990; Dohi et al., 1990). The novel compound NO-1886, has been reported to increase lipoprotein lipase enzyme mass in postheparin plasma, and lipoprotein lipase mRNA and lipoprotein lipase activity in epididymal adipose tissue and myocardium, causing a reduction in plasma triglyceride levels with concomitant elevation of high density lipoprotein (HDL) cholesterol by increasing lipoprotein lipase activity in normal and diabetic rats (Tsutsumi et al., 1993, 1995). Furthermore, NO-1886 activates lipoprotein lipase in primary cultured adipose and skeletal muscle cells in vitro (Hagi et al., 1997). Long-term administration of NO-1886 also protects against the development of experimental atherosclerosis in rats (Tsutsumi et al., 1993) and rabbits (Chiba et al., 1997). The present study was

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designed to determine whether NO-1886 ameliorates the aging-related deterioration of endothelium-dependent relaxation, an early event in the atherosclerotic process.

2. Materials and methods

2.1. Animal experiments

NO-1886, 4-diethoxyphosphorylmethyl-*N*-(4-bromo-2-cyanophenyl) benzamide was synthesized in the New Drug Research Laboratory of Otsuka Pharmaceutical Factory, Naruto, Tokushima, Japan. Histamine, sodium nitroprusside and phenylephrine were obtained from Sigma, St. Louis, MO. All other chemicals used were the high grade commercially available products. Seven-month-old male Sprague–Dawley rats (Old Control group) weighing 500–700 g were obtained from Japan SLC, (Shizuoka, Japan). The animals were maintained under a 12-h light–dark cycle at a constant temperature of 23 ± 2°C. Rats were fed either rat chow alone (CRF-1, Oriental Yeast, Tokyo, Japan) or chow containing NO-1886 (at a concentration to give 50 mg/kg body weight of NO-1886) for 3 months (Old + NO-1886 group). The animals were allowed ad libitum access to food and tap water. Food consumption was measured daily, and body weight was recorded weekly. Four hours after food removal, the animals were killed by exsanguination under sodium pentobarbital anesthesia. Blood samples were collected from the posterior vena cava for lipid measurement. The thoracic aorta was removed immediately for measurement of vasorelaxation responses. A portion of the aorta was fixed in 10% neutral buffered formalin, and embedded in paraffin. Thin sections (4 μm) were stained with hematoxylin and eosin. The aorta on the sections were examined histopathologically. Two-month-old male Sprague–Dawley rats (Young-rat group) were used for comparison.

2.2. Analytical methods

2.2.1. Plasma lipids, glucose, insulin, superoxide dismutase and lipid peroxide

Plasma total cholesterol (cholesterol C-test, Wako Pure Chemical Industries, Osaka, Japan), HDL cholesterol (Nescote HDL-C kit N, Nippon Shoji, Osaka, Japan), triglycerides (triglyceride G-test, Wako), and glucose (glucose CII test, Wako) were determined by conventional enzymatic methods. Insulin was determined by conventional enzyme immunoassay, with the use of the Glazyme insulin-EIA test (Wako). Superoxide dismutase (SOD) was determined by means of the SOD-test Wako (Wako) with the nitro blue tetrazolium method (McCord and Fridovich, 1969). Lipid peroxide was determined with the LPO-test Wako (Wako) by the method of Yagi (1976).

2.2.2. Tension measurements of thoracic aorta

The thoracic aorta between the aortic arch and the diaphragm was dissected free following exsanguination and cut into 2–3 mm rings. The rings were mounted isometrically at a resting tension of 1.0 g in a 5-ml organ bath (VFER micro easy magnus, Medical Kishimoto, Kyoto, Japan) containing a Krebs solution with the following composition: NaCl 118.4 mM, KCl 4.9 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 1.2 mM, $NaHCO_3$ 25.0 mM, KH_2PO_4 1.2 mM, glucose 11.1 mM and ascorbic acid 1.2 mM. The medium was maintained at 34°C and bubbled with 95% O_2 and 5% CO_2. The arteries were contracted with phenylephrine at a concentration corresponding to the EC_{80} values (10^{-6} M). Histamine, acetylcholine and sodium nitroprusside were added cumulatively. Relaxation responses were measured with an isometric transducer (AP-5, Medical Kishimoto) and expressed as percentages of the phenylephrine-induced contraction.

2.3. Statistical analysis

The results are expressed as means $\pm$ S.E.M. Comparisons were by analysis of variance followed by Fischer least squares post-hoc tests or by Student's *t*-test or Aspin–Welch's *t*-test where the data were not normally distributed.

3. Results

3.1. Effects of NO-1886 on body weight and food consumption

Old rats were much heavier (mean starting weight 578 g) than young rats (mean 290 g). There were no differ-

Table 1
Plasma levels of lipids, glucose and insulin in old rats treated with NO-1886

	n	Lipids (mg/dl)			Glucose (mg/dl)	Insulin (pg/ml)
		Total cholesterol	HDL-cholesterol	Triglyceride		
Old rats						
Control	(9)	98 ± 4	72 ± 6	233 ± 10	194 ± 6	67.0 ± 8.6
NO-1886	(10)	173 ± 5^{a}	142 ± 6^{a}	172 ± 16^{b}	185 ± 6	68.8 ± 9.5
Young rats	(7)	65 ± 1^{a}	60 ± 2	151 ± 13^{a}	128 ± 3^{a}	23.1 ± 5.7^{b}

Data are expressed as means $\pm$ S.E.M. Significant difference from the respective values in old control rats: $^{a}P < 0.001$, $^{b}P < 0.01$.

ences in initial body weight between the Old Control group and Old + NO-1886 groups, however weight gain was slightly lower with NO-1886 treatment (17 ± 5 g vs. 33 ± 5 g in the Old Control group, $P = 0.05$). Food intake was measured in the old rat groups at 6 and 7 weeks of treatment. There were no significant differences between the Old Control and Old + NO-1886 groups (data not shown).

3.2. Effects of NO-1886 on plasma lipids, glucose, insulin, superoxide dismutase and lipid peroxide

Table 1 shows the effects of NO-1886 on plasma lipids, glucose and insulin. The plasma levels of total cholesterol, triglycerides, glucose and insulin were significantly higher in the Old Control group than in young rats (Young-rat group). The plasma HDL-cholesterol level was not altered significantly with age. Administration of NO-1886 decreased plasma triglyceride levels with concomitant increases in plasma HDL cholesterol level. The increase in plasma total cholesterol concentrations in NO-1886-treated old rats was due to the increase in plasma HDL-cholesterol level. NO-1886 had no effects on plasma levels of glucose or insulin (Table 1). We also measured plasma lipid peroxide (Old Control, 5.34 ± 0.91 nmol/ml; Old + NO-1886, 5.26 ± 0.69 nmol/ml), and superoxide dismutase (Old Control, $10.1 \pm 2.2\%$; Old + NO-1886, $9.2 \pm 3.0\%$). They were not significantly different.

3.3. Effects of NO-1886 on relaxation responses

Figs. 1–3 show the dose–response curves for endothelium-dependent and -independent relaxation. The endothelium-dependent relaxation by histamine was significantly impaired in the Old Control group compared to the Young-rat group, whereas the endothelium-independent relaxation by sodium nitroprusside remained unchanged. The endothelium-dependent relaxation of the aorta by histamine in the Old + NO-1886 group was sufficiently improved compared to that of the Old Control group (Fig. 1) so as

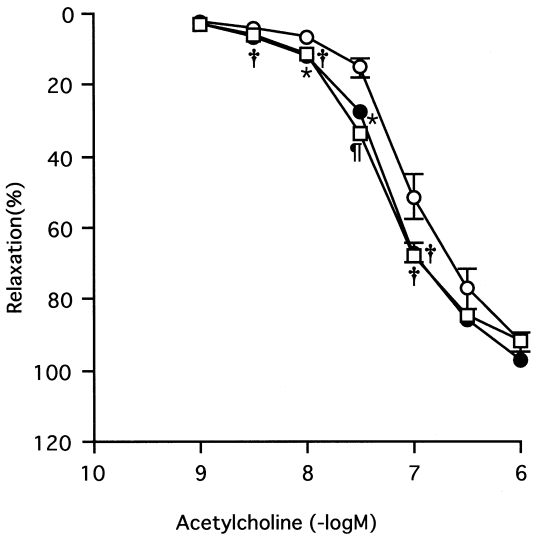
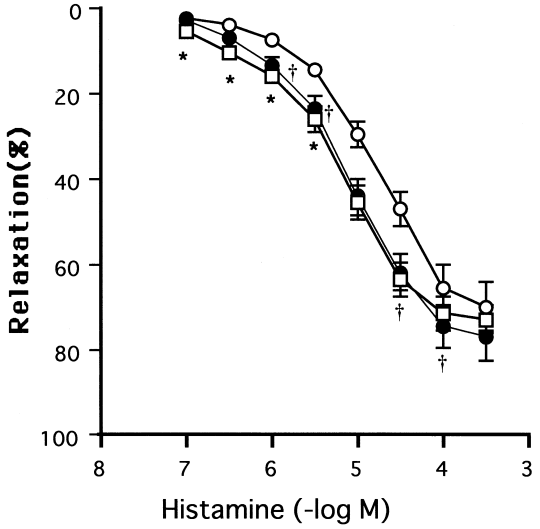


Fig. 2. Effects of NO-1886 on the dilator response of rat thoracic aorta to acetylcholine. The aortas were contracted with the EC_{80} concentration of phenylephrine. Dilator responses are expressed as percentage of phenylephrine-induced contraction. ○; Old control rats, ●; Old + NO-1886 treated old rats, □; Young rats. Data are expressed as means ± S.E.M. ($n = 7$–10). Significantly different from the respective values in old control rats: $^{¶}P < 0.001$, $* P < 0.01$, $^{†}P < 0.05$.



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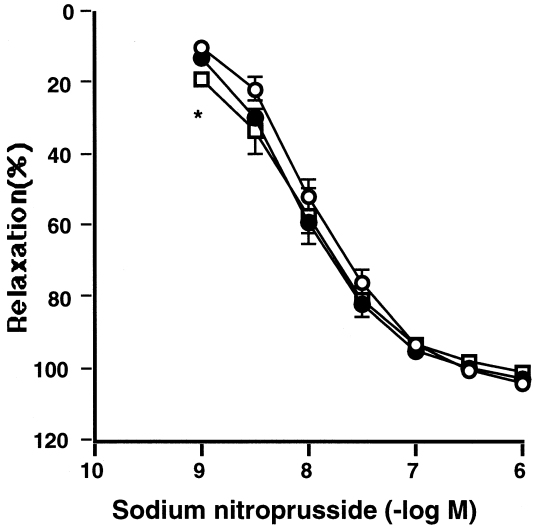

Fig. 3. Effects of NO-1886 on the dilator response of rat thoracic aorta to sodium nitroprusside. The aortas were contracted with the EC_{80} concentration of phenylephrine. Dilator responses are expressed as percentage of phenylephrine induced contraction. ○; Old control rats, ●; Old + NO-1886 treated old rats, □; Young rats. Data are expressed as means ± S.E.M. ($n = 7$–9). Significantly different from the respective values in old control rats: $* P < 0.05$.

Table 2
EC_{50} for the dose-dependent relaxation

	n	EC_{50}		
		Histamine (mmol/l)	Acetylcholine (nmol/l)	Sodium nitroprusside (nmol/l)
Old rats				
Control	(7)	139.3 ± 110.6	127.0 ± 46.0	9.8 ± 1.7
NO-1886	(7)	67.2 ± 49.8	58.8 ± 3.4	7.8 ± 1.0
Young rats	(7)	17 ± 10.0	53.4 ± 1.1	7.4 ± 1.4

Data are expressed as means ± S.E.M.

not to be different from that of young rats. NO-1886 also improved endothelium-dependent relaxation of the aorta induced by acetylcholine (Fig. 2). In contrast, NO-1886 showed no effect on the endothelium-independent relaxation by sodium nitroprusside, a donor of nitric oxide (Fig. 3). Table 2 shows the ED_{50} of the dose-dependent relaxation. There was no significant difference in each group.

3.4. Histological findings of aorta

Histological examination showed no evidence of atherosclerotic lesions in the aorta of any of the old rats (data not shown).

4. Discussion

Aging is associated with progressive development of dyslipidemias, insulin resistance and obesity, all risk factors for cardiovascular disease and atherosclerosis (Austin, 1991; Reaven, 1988; Hubert et al., 1983). NO-1886 is a novel lipoprotein lipase activator that has already been shown to reduce plasma triglyceride levels with concomitant elevation of HDL cholesterol levels (Tsutsumi et al., 1993) and reduce obesity induced by high-fat feeding (Kusunoki et al., 1997). The results of the present study demonstrated, in addition to these effects of NO-1886, a clear amelioration of the aging-related deterioration of endothelium-dependent relaxation. Endothelium-derived relaxing factor is now found to nitric oxide (Ignarro et al., 1987, Palmer et al., 1987). The improvement in the endothelium-dependent relaxation by NO-1886 may be due to improvement of the production of nitric oxide by the endothelium, while the effect of nitric oxide on smooth muscle cells was not altered.

Damage to endothelial cells has been suggested as a potent trigger for the onset of coronary atherosclerosis and coronary vasospasm (Vanhoutte et al., 1986). Cohen et al. (1988) reported that impairment of endothelial function exists before histological findings of atherosclerosis become apparent. In the present study, the endothelium-dependent relaxation by histamine was significantly reduced with aging, although the histopathological examination of the aorta in the old rats did not show atherosclerotic lesions.

The mechanism for the protective effect of NO-1886 on the aortic endothelium was not clear from the present results. However, both increased plasma triglycerides (Fontbonne et al., 1989) and decreased HDL cholesterol (Badimon et al., 1990) are associated with development of atherosclerosis and it is tempting to speculate that it is the ameliorating effect of NO-1886 on these variables that might account for the observed benefits. Insulin resistance is also associated with development of cardiovascular disease. The elevated basal insulin and glucose values in the untreated old rats is suggestive of insulin resistance, however, NO-1886 had no effect on either variable. NO-1886 also has no antioxidant properties.

In summary, NO-1886 prevented the impairment of endothelium-dependent relaxation of rat thoracic aorta associated with aging, possibly by correcting the lipid abnormalities of aging. Therefore, the compound is potentially beneficial for the treatment of hyperlipidemia, which is commonly associated with aging.

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