European Journal of Pharmacology 599 (2008) 102–109

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular Pharmacology

Cilostazol improves endothelial dysfunction by increasing endothelium-derived hyperpolarizing factor response in mesenteric arteries from Type 2 diabetic rats

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ARTICLE INFO

Article history:
Received 24 June 2008
Received in revised form 18 September 2008
Accepted 5 October 2008
Available online 10 October 2008

Keywords:
Cilostazol
Diabetes
Endothelium-derived hyperpolarizing factor
Endothelial dysfunction
Mesenteric artery

ABSTRACT

Diabetes mellitus impairs endothelial function, an effect that can be considered a hallmark of the development of cardiovascular diseases in diabetics. Cilostazol, a selective phosphodiesterase 3 inhibitor, is currently used to treat patients with diabetic vascular complications. However, the effects of cilostazol on responses mediated by endothelium-derived relaxing [in particular, nitric oxide (NO) and hyperpolarizing factors (EDHF)] and contracting factors remain unclear. Here, we hypothesized that cilostazol could improve endothelial dysfunctions in mesenteric arteries isolated from type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Using cilostazol-treated (100 mg/kg/day for 4 weeks) or -untreated OLETF and control (Long Evans Tokushima Otsuka) rats, we examined the acetylcholine-induced endothelium-dependent responses and the cell-permeant cyclic adenosine monophosphate (cAMP) analog-induced relaxations in the superior mesenteric artery. We also determined blood parameters in these animals. In OLETF rats, chronic treatment with cilostazol reduced the blood levels of triglyceride, non-esterified fatty acids, and leptin, and increased antioxidant capacity, but did not alter the blood glucose or insulin levels. In studies on mesenteric arteries from cilostazol-treated OLETF animals, the cilostazol treatment improved: (a) the acetylcholine-induced EDHF-mediated relaxation and (b) the cAMP-mediated relaxation. However, cilostazol did not alter the NO-mediated relaxation or the endothelium-derived contracting factor-mediated contraction. These results suggest that cilostazol improves endothelial functions in OLETF mesenteric arteries by increasing EDHF signaling, and that it normalizes some metabolic abnormalities in OLETF rats. On that basis, cilostazol may prove to be a potent drug for the clinical treatment of diabetic vasculopathy.

1. Introduction

Cilostazol, 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3, 4-dihydro-2(1H)-quinolinone, increases the intracellular cyclic adenosine monophosphate (cAMP) level by inhibiting the activity of phosphodiesterase 3 (Lugnier, 2006). Cilostazol is currently used in the treatment of intermittent claudication in diabetic patients (Dawson et al., 1998) and for peripheral vascular occlusive diseases (Kwon et al., 2005). Cilostazol has also been shown to be effective in preventing both silent brain infarction in Japanese patients with type 2 diabetes (Shinoda-Tagawa et al., 2002) and atherosclerosis (Meadows and Bhatt, 2007), effects considered to result from its anti-platelet, vasodilator, and anti-proliferative actions. Although there is an accumulating body of evidence to show that cilostazol has beneficial effects on several pathophysiological states, the effects of this drug on endothelium-dependent vasomotor responses in type 2 diabetes remain uncertain.

Macro- and micro-vascular diseases are the principal contributors to the increased morbidity and mortality associated with both type 1 and type 2 diabetes [indeed, type 2 diabetic patients have a mortality rate 3–4 times that of the general population (Haffner et al., 1998)]. Endothelial dysfunction may play a key role in the development of both macro- and micro-angiopathy both in diabetic patients and in animal models of diabetes (De Vriese et al., 2000; Matsumoto et al., 2006a; Pieper, 1998). Evidence is accumulating to indicate (a) that endothelium-dependent relaxation is impaired in several blood vessels in type 2 diabetes [both in animal models and in patients (De Vriese et al., 2000; Kobayashi et al., 2004; Van Gaal et al., 2006)] as well as in type 1 diabetes (De Vriese et al., 2000; Hattori et al., 1991; Kamata et al., 1989; Matsumoto et al., 2003, 2005a, 2007c; Mayhan and Patel, 1998), and (b) that a decreased production of endothelium-derived relaxing factors [in particular, nitric oxide (NO) and hyperpolarizing factors (EDHF)] and/or defects in endothelium-derived relaxing factors-signaling may underlie the impairment of endothelium-dependent relaxation seen in type 2 diabetic vessels (De Vriese et al., 2000; Ding and Triggle, 2005). Moreover, this impairment of vascular relaxation may be attributable not only to reduced amounts of endothelium-derived relaxing factor and/or EDHF, but also to increased amounts of endothelium-derived contracting factor, leading to diabetic vasculopathy (Cohen, 2005; De Vriese et al., 2000; Feletou and Vanhoutte, 2006; Matsumoto et al., 2006c). Although the balance between endothelium-derived relaxing factors and endothelium-

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doi:10.1016/j.ejphar.2008.10.006

derived contracting factors is known to play an important role in setting vascular tone in both physiological and pathophysiological states, little information is available as to whether (and to what extent) manipulation of such factors by drugs might be able to improve vasculopathy in type 2 diabetes.

Otsuka Long-Evans Tokushima Fatty (OLETF) rats are characterized by an early increase in serum insulin, and also by late-onset hyperglycemia, mild obesity, and mild type 2 diabetes (Kawano et al., 1992), and there are several reports of abnormalities of vascular function in this diabetic model (Kagota et al., 2000; Matsumoto et al., 2006b,c, 2007b). Moreover, we recently demonstrated: (a) that endothelial dysfunction is present in the mesenteric arteries of aged OLETF rats, (b) that this may result from an imbalance between endothelium-derived factors (reduced endothelium-derived relaxing factors signaling and increased endothelium-derived contracting factors signaling), (c) that the mechanisms underlying this abnormality may involve increments in both cyclooxygenase-1 and -2 activities (Matsumoto et al., 2007a), and (d) that the impairment of EDHF-type relaxation seen in this diabetic rat may be attributable not only to a reduction in cAMP/protein kinase A signaling, but also to reduced endothelial Ca^{2+}-activated potassium channel (K_{Ca}) activities (Matsumoto et al., 2006c). Although there is considerable evidence to show that cilostazol has beneficial effects on several pathophysiological states, no study has yet investigated the effects of chronic cilostazol treatment on the endothelial dysfunction present in type 2 diabetic mesenteric arteries. Therefore, in the present study we carried out just such an investigation, using mesenteric arteries isolated from OLETF rats.

2. Materials and methods

2.1. Reagents

Apamin, phenylephrine, indomethacin, N^G-nitro-L-arginine (L-NNA), N^6, O^2-dibutyryl-adenosine-3′, 5′-cyclic monophosphate (db-cAMP), and TRAM34 [1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole] were all purchased from Sigma Chemical Co. (St. Louis, MO, USA), while acetylcholine chloride was purchased from Daiichi-Sankyo Pharmaceuticals (Tokyo, Japan). Drugs were dissolved in saline, except for TRAM34 (dissolved in dimethyl sulfoxide) and indomethacin. Indomethacin was dissolved first in a small amount of 0.1 M Na_2CO_3 solution, and then made up to the final volume with distilled water.

2.2. Animals and experimental design

Five-week-old male rats [OLETF rats and Long-Evans Tokushima Otsuka (LETO) rats, a genetic control for OLETF] were supplied by the Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). Food and water were given ad libitum in a controlled environment (room temperature 21–22 °C, room humidity 50±5%) until the rats were 36–42 weeks old. Some OLETF and LETO rats were chronically given cilostazol (100 mg/kg/day, p.o.) for 4 weeks starting at 36–42 weeks old. Thus, we studied four groups: cilostazol-untreated LETO and OLETF groups and cilostazol-treated LETO and OLETF groups. This study was approved by the Hoshi University Animal Care and Use Committee, and all studies were conducted in accordance with "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health, and "Guide for the Care and Use of Laboratory Animals" adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology, Japan).

2.3. Measurement of blood glucose, cholesterol, triglyceride, insulin, leptin, antioxidant, and non-esterified fatty acid, and blood pressure

Plasma parameters and systemic blood pressure were measured as described previously (Matsumoto et al., 2003, 2004, 2005a,b, 2007c). Briefly, plasma glucose, cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol, and serum non-esterified fatty acid levels were each determined by the use of a commercially available enzyme kit (Wako Chemical Company, Osaka, Japan). Plasma insulin was measured by enzyme-immunoassay (Shibayagi, Gunma, Japan). Plasma leptin was determined by enzyme-linked immunosorbent assay (Morinaga Institute of Biological Science, Yokohama, Japan). The plasma antioxidant level was determined by the use of a commercially available kit (Cayman Chemical, Ann Arbor, MI, USA). This Trolox equivalent antioxidant-capacity assay is based on the scavenging of the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical [ABTS(*)] converting it into a colorless product. After a given rat had been in a constant-temperature box at 37 °C for a few minutes, its blood-pressure was measured by the tail-cuff method using a blood pressure analyzer (BP-98A; Softron, Tokyo, Japan).

2.4. Measurement of isometric force

Vascular isometric force was recorded as in our previous papers (Matsumoto et al., 2003, 2005a, 2006c, 2007a). At 40–46 weeks of age, rats were anesthetized with diethyl ether, then euthanized by decapitation. The superior mesenteric artery was rapidly removed and immersed in oxygenated, modified Krebs-Henseleit solution (KHS). This solution consisted of (in mM) 118.0 NaCl, 4.7 KCl, 25.0 $NaHCO_3$, 1.8 $CaCl_2$, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, and 11.0 dextrose. The artery was carefully cleaned of all fat and connective tissue, and ring segments 2 mm in length were suspended by a pair of stainless-steel pins in a well-oxygenated (95% O_2- 5% CO_2) bath containing 10 mL of KHS at 37 °C. The rings were stretched until an optimal resting tension of 1.0 g was loaded, and then allowed to equilibrate for at least 60 min. Force generation was monitored by means of an isometric transducer (model TB-611T; Nihon Kohden, Tokyo, Japan).

For the relaxation studies, mesenteric rings were precontracted with an equally effective concentration of phenylephrine (100 nM–3 μM) (i.e., so that the tension developed in response to phenylephrine was similar among all groups). There was no significant difference in the response to phenylephrine among the LETO (n=32), OLETF (n=32), cilostazol-treated LETO (n=32), and cilostazol-treated OLETF (n=32) groups (1.65±0.05 g, 1.68±0.05 g, 1.63±0.04 g, and 1.69±0.05 g, respectively). When the phenylephrine-induced contraction had reached a plateau level, acetylcholine (1 nM–10 μM) was added in a cumulative manner. After the addition of sufficient aliquots of the agonist to produce the chosen concentration, a plateau response was allowed to develop before the addition of the next dose of the same agonist. To investigate the influences of the various factors that might constitute endothelium-derived relaxing factor in the present preparations, we examined acetylcholine-induced relaxation in the absence or presence of various inhibitors, as follows: 1) 10 μM indomethacin plus 10 μM TRAM34 (specific inhibitor of the intermediate-conductance K_{Ca} channel) plus 100 nM apamin (specific inhibitor of the small-conductance K_{Ca} channel) (to investigate NO-mediated relaxation), 2), 10 μM indomethacin plus 100 μM L-NNA (to investigate EDHF-type relaxation). Rings were incubated with the appropriate inhibitor(s) for 30 min before administration of phenylephrine. To investigate the cAMP-mediated relaxation, db-cAMP (a cell-permeable cAMP analog) (1 μM–100 μM) was added in a cumulative manner in the presence of 10 μM indomethacin plus 100 μM L-NNA.

For the contraction studies, mesenteric rings were first contracted using 80 mM K^+, these responses being taken as 100%. There was no significant difference in the response to 80 mM K^+ among the LETO (n=8), OLETF (n=8), cilostazol-treated LETO (n=8), and cilostazol-treated OLETF (n=8) groups (1.54±0.03 g, 1.57±0.06 g, 1.60±0.04 g, and 1.54±0.03 g, respectively). To investigate the endothelium-derived contracting factor-mediated response, mesenteric rings were treated with 100 μM L-NNA for 30 min. After this incubation period, acetylcholine (10 nM–10 μM) was cumulatively applied.