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Abnormalities of endothelium-dependent responses in mesenteric arteries from Otsuka Long-Evans Tokushima Fatty (OLETF) rats are improved by chronic treatment with thromboxane A_2 synthase inhibitor

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ABSTRACT

Thromboxane A_2 (TXA_2) is thought to contribute to the development of diabetic complications. We tested the hypothesis that the impaired endothelial function seen in Otsuka Long-Evans Tokushima Fatty (OLETF) rats (a type 2 diabetic model) might be improved by chronic treatment with ozagrel, a TXA_2 synthase inhibitor. In mesenteric arteries from OLETF rats (40–46 weeks old) [vs. those from age-matched Long-Evans Tokushima Otsuka (LETO) rats]: (1) ACh-induced endothelium-dependent relaxation, NO-mediated relaxation, and endothelium-derived hyperpolarizing factor (EDHF)-type relaxation were all reduced; (2) ACh-induced cyclooxygenase-dependent contraction was enhanced; (3) endothelium-derived contracting factor (EDCF)-mediated contraction was enhanced; (4) ACh-stimulated nitrite production was reduced but the nitrate/nitrite ratio was increased; and (5) ACh-stimulated production of TXA_2 was increased. Chronic treatment with ozagrel (100 mg/kg/day for 4 weeks, starting when they were 36–42 weeks of age) partly corrected the above abnormalities. These results suggest that ozagrel has normalizing effects on endothelial functions in OLETF mesenteric arteries, at least partly by increasing endothelium-derived relaxing factors (i.e., NO and EDHF) signaling and reducing EDCF signaling.

1. Introduction

Sixteen million Americans currently have type 2 diabetes, and vascular complications are the commonest causes of morbidity and mortality in diabetic patients. These vascular abnormalities include alterations in blood vessel contraction and relaxation (vascular reactivity), which have been detected both in type 2 diabetic patients and in animal models [1,2].

Normal vascular endothelial function depends on a controlled balance between the production/release of endothelium-derived relaxing (EDRF) and contracting (EDCF) factors. Indeed, the dilator influences exerted by endothelium-derived nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF) are normally in harmony with the release of constrictors such as endothelin, prostaglandins (PGs), and thromboxane (TX) A_2 [3–6]. A marked imbalance toward an increased release and/or functional impact of EDCFs on vascular function is associated with established endothelial dysfunction, a hallmark both of certain cardiovascular diseases (such as atherosclerosis, diabetes, and hypertension) and of aging [4,6].

TXA_2, a lipid mediator originating from arachidonic acid metabolism through the cyclooxygenase (COX) pathway, is a powerful constrictor of vascular smooth muscle. Studies have shown that vascular production of TXA_2 may be increased in several cardiovascular diseases, and that its signaling plays an important role in the development of such diseases [4,6–10]. Inhibition of TXA_2 signaling has been reported to improve a number of cardiovascular diseases [8–10], and we wondered whether manipulation of TXA_2 signaling by chronic treatment with a TXA_2 synthase (TXS) inhibitor might be able to improve endothelial dysfunction in type 2 diabetes.

OLETF rats are characterized by an early increase in serum insulin, and also by late-onset hyperglycemia, mild obesity, and mild type 2 diabetes [11], and abnormalities of vascular function have been shown to be present in this diabetic model [2]. Recently, we found: (a) that endothelial dysfunction is present in the mesenteric arteries of OLETF rats, (b) that this may result from an imbalance between opposing endothelium-derived factors (reduced EDRFs signaling and increased EDCFs signaling), and (c) that the mechanisms underlying this abnormality may involve increments in COX activities [12]. The purpose of the present study was to test the hypothesis that chronic treatment of type 2 diabetic OLETF rats with a TXS inhibitor, ozagrel, would normalize endothelial functions in their mesenteric arteries.

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2. Materials and methods

An expanded Section 2 is available in the online-only Data Supplement.

2.1. Reagents

Reagents were from Sigma unless otherwise stated.

2.2. Animals and experimental design

Five-week-old male rats (OLETF rats and LETO rats, a genetic control for OLETF) were supplied by the Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). Some OLETF and LETO rats were chronically given ozagrel (100 mg/kg/day, p.o.) for 4 weeks starting at 36–42 weeks old. Thus, we studied four groups: ozagrel-untreated LETO and OLETF groups and ozagrel-treated LETO and OLETF groups. All experimental protocols were approved by the local Ethics Committee.

2.3. Measurement of blood glucose, cholesterol, triglyceride and insulin, and blood pressure

Plasma parameters and systemic blood pressure were measured as described previously [2,12–14].

2.4. Measurement of isometric force

Vascular isometric force was recorded as in our previous papers [12–14]. For the relaxation studies, when the PE-induced precontraction had reached a plateau level (Supplemental Table 1), ACh was added in a cumulative manner. We examined such ACh-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 the EDHF-type relaxation), and (3) 100 μM L-NNA plus 10 μM TRAM34 plus 100 nM apamin (to investigate the COX-mediated response). Rings were incubated with the appropriate inhibitor(s) for 30 min before administration of PE. To investigate the EDCF-mediated contractile response, mesenteric rings were treated with 100 μM L-NNA for 30 min, and then ACh was cumulatively applied. This EDCF-mediated response was expressed as a percentage of the 80 mM K^+-induced contraction in the same preparation.

2.5. Measurement of nitrite (NO_2^-) and nitrate (NO_3^-)

The concentrations of nitrite and nitrate in the effluent from each tissue were measured by the method described previously [15]. Each mesenteric ring was placed in KHS at 37 °C, and then treated with ACh (10 μM) for 15 min. The concentrations of nitrite and nitrate in the KHS were measured using an automated NO detector/high-performance liquid chromatography system (ENO20; Eicom, Kyoto, Japan).

2.6. Release of prostaglandins

Prostanoid release was measured as in our previous papers [12,14]. Each mesenteric ring was placed for 30 min in KHS at 37 °C, and then 10 μM ACh was applied for 15 min. The prostaglandins in the KHS were measured using a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI).

2.7. Western blotting

COX and eNOS protein levels were quantified using immunoblotting procedures, essentially as described before [12,14]. Mesenteric arterial protein extracts (20 μg) were applied to 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Blots were incubated with the appropriate antibody [anti-COX1 (70 kDa; 1:500), anti-COX2 (72 kDa; 1:500), or anti-eNOS (140 kDa; 1:1000)], with anti-β-actin (42 kDa; 1:5000) antibody being used for normalization. Detection involved the use of a horseradish peroxidase-conjugated IgG followed by enhanced chemiluminescence. Band intensity was quantified by densitometry.

2.8. Statistical analysis

Data are expressed as means ± SE. Each relaxation response is expressed as a percentage of the contraction induced by PE. Contractile responses are expressed as a percentage of the response to 80 mM KCl. When appropriate, statistical differences were assessed by Dunnett's test for multiple comparisons after a one-way analysis of variance (ANOVA), a probability level of $P \leq 0.05$ being regarded as significant. Statistical comparisons between concentration–response curves were made using a two-way ANOVA, with Bonferroni's correction for multiple comparisons being performed post hoc ($P \leq 0.05$ again being considered significant).

3. Results

3.1. General parameters

As shown in Table 1, the non-fasted blood glucose concentrations in OLETF rats were significantly higher than those of the LETO rats (also non-fasted). The body weight, the plasma insulin and triglyceride levels, and the systolic blood pressure were all significantly higher in OLETF rats than in LETO rats, while total cholesterol and HDL levels and heart rate were similar between the two groups. Treatment with ozagrel did not alter the above parameters in OLETF rats. In LETO rats, treatment with ozagrel significantly lowered body weight (vs. non-treated LETO rats).

3.2. Endothelium-dependent relaxation and contraction

When the PE-induced contraction had reached a plateau, the level of which was similar among the four groups (Supplemental Table 1), the relaxation response to ACh was examined (Fig. 1). ACh induced a concentration-dependent relaxation (with the maximum response being at 100–300 nM, and responses then being progressively weaker up to 10 μM), and this relaxation was significantly weaker in rings from OLETF rats than in those from LETO rats (Fig. 1A). In LETO rats, ozagrel treatment caused no significant alteration in the relaxation (vs. the untreated LETO group) (Fig. 1B). In the OLETF group, however, ozagrel significantly increased the relaxation response to one of the lower concentrations of ACh (i.e., 10 nM) and tended to increase those to the other ACh concentrations (Fig. 1C).

To investigate which (if any) EDRFs might be affected in mesenteric arteries from ozagrel-treated OLETF rats, we examined the ACh-induced relaxation in the presence of various inhibitors (Figs. 2–4). To investigate the NO-mediated relaxation, we investigated ACh-induced relaxation in the combined presence of 10 μM indomethacin, 100 nM apamin, and 10 μM TRAM34 (Fig. 2). Under these conditions [in which, the PE-induced contraction was similar among the four groups (Supplemental Table 1)], the NO-mediated relaxation was slightly, but significantly, weaker in rings from OLETF rats than in those from LETO rats (Fig. 2A). In both LETO