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Sarpogrelate protects against high glucose-induced endothelial dysfunction and oxidative stress

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ABSTRACT

This study was designed to investigate the effect of sarpogrelate hydrochloride on impaired endothelium-dependent relaxation (EDR) induced by high glucose in isolated rat aorta. Both acetylcholine-induced EDR and sodium nitroprusside-induced endothelium-independent relaxation (EIR) were measured after the rings were exposed to high glucose in the absence and presence of sarpogrelate hydrochloride. Co-incubation of aortic rings with high glucose for 24 h resulted in a significant inhibition of EDR, but had no effects on EIR. After incubation of the rings in the co-presence of sarpogrelate hydrochloride with high glucose for 24 h, sarpogrelate hydrochloride significantly attenuated impaired EDR. This protective effect of sarpogrelate hydrochloride was abolished by N^G-nitro-L-arginine methyl ester. Sarpogrelate hydrochloride significantly decreased superoxide anion (O_2^-) production and increased superoxide dismutase (SOD) activity and the nitric oxide (NO) release. These results suggest that sarpogrelate hydrochloride can restore impaired EDR induced by high glucose in isolated rat aorta, which may be related to scavenging oxygen free radicals and enhancing NO production.

1. Introduction

Endothelial barrier dysfunction plays a pivotal role in the pathogenesis of diabetic vascular complications [1]. Serotonin (5-hydroxytryptamine, 5-HT), a major non-peptidergic substance released from activated platelets, mediates vasoconstriction and induces the activation of other platelets, which may promote coronary events by adhering to unstable atherosclerotic plaque and initiating thrombotic complications [2,3]. Serum 5-HT concentrations are elevated in diabetes [4,5], suggesting that it may be involved in the development of diabetic complications, although there is no clear clinical evidence for its involvement in the pathogenesis of diabetic complications. Recent studies have revealed that 5-HT has produced both contraction and relaxation of the vascular smooth muscles [6–9]. Multiple 5-HT receptors are involved in mediating these effects. 5-HT-induced vasoconstrictions of arteries are mainly mediated by 5-HT_{2A} receptor subtype [10]. In porcine coronary and pulmonary artery, 5-HT causes endothelium-dependent relaxation (EDR) responses [11–14]. A selective 5-HT_{2A} receptor antagonist (sarpogrelate) was introduced as a therapeutic agent for the treatment of ischemic diseases associated with thrombosis [15]. Sarpogrelate hydrochloride inhibits thrombus formation [16,17], suppresses platelet aggregation [18,19], inhibits 5-HT induced coronary artery spasm [20] and contraction of coronary artery in the porcine model mediated by 5-HT and α-methylserotonin [21] and also inhibits vascular smooth muscle cell proliferation [22].

High glucose in vitro or in vivo has been reported to inhibit acetylcholine (ACh)-mediated endothelium-dependent relaxation responses [23], to impair the biological synthesis pathway of nitric oxide (NO) [24], and to generate reactive oxygen species [25]. However, it is unclear whether sarpogrelate hydrochloride can improve impaired EDR evoked by high glucose. In the present study, we sought to examine whether sarpogrelate hydrochloride exerts beneficial effect on high glucose-induced endothelial dysfunction in isolated rat aorta.

2. Materials and methods

2.1. Drugs and chemicals

Sarpogrelate hydrochloride was obtained from Mitsubishi Chemical Corporation (Tokyo, Japan). ACh, sodium nitroprusside (SNP), N^G-nitro-L-arginine methyl ester (L-NAME), phenylephrine, L-arginine, and superoxide dismutase (SOD) were purchased from Sigma Chemical Co (Saint Louis, Mo, USA). The rest of drugs were prepared in bidistilled water and diluted with Krebs buffer immediately before the experiment.

2.2. Organ chamber experiment

Adult Sprague-Dawley rats of both genders (body weight: 180–200 g), which were supplied by the Medical Experimental Animal Center of Harbin Medical University,

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China, were used in accordance with the Guidelines on the Care and Use of Laboratory Animals issued by the Chinese Council on Animal Research and the Guidelines of the Animal Care. The study was approved by the ethical committee of Harbin Medical University.

The rats were killed by exsanguination under anesthesia with pentobarbital sodium (30 mg/kg). The thoracic aorta was removed and placed immediately in Krebs bicarbonate buffer of the following composition (in mM): NaCl 118.3; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25.0; glucose 5.5. The aortic segment was cut into 3–4 mm rings. The adhering perivascular tissue was carefully removed. The rings were suspended horizontally between two stainless-steel stirrups in organ chambers filled with 5 mL Krebs' solution at 37 °C, aerated continuously with a mixture of 95% O_2 and 5% CO_2. One stirrup was connected to an anchor and the other to a force transducer for recording of isometric tension. Rings were equilibrated for 90 min under 2 g resting tension. Then the rings were contracted with a submaximal concentration of phenylephrine (1 μM). After reaching a stable contraction plateau (approximately 15 min), rings were relaxed with cumulative concentrations of ACh (0.03–3 μM). The endothelium was considered to be intact when ACh caused at least a 75% relaxation of rings.

2.3. Experimental protocol

Rings were incubated with normal (5.5 mM) and high concentrations (11 mM or 25 mM) of glucose for 24 h. In the sarpogrelate hydrochloride groups, rings were incubated with various concentrations of sarpogrelate hydrochloride (0.1–10 μM) plus high glucose (25 mM) for 24 h, respectively. To investigate whether increased production of superoxide anion and decreased NO synthesis exert detrimental effects and whether oxygen free radicals and enhanced NO production contribute to the protective effect of sarpogrelate hydrochloride, some rings were co-incubated in high glucose in the presence of SOD (200 U/mL), L-arginine (3 mM) or D-arginine (3 mM) for 24 h, the others were incubated with L-NAME (10 μM) for 24 h. We also compared the effects of SOD, L-arginine, or D-arginine alone or in a combination of SOD and L-arginine on EDR of aortic rings with those of sarpogrelate.

After the above incubations periods, all rings were washed repeatedly and then recontracted with phenylephrine (1 μM) and relaxation responses to ACh (0.03–3 μM) were repeated. Before finishing the experiment, relaxation to SNP at the plateau phase of the phenylephrine contraction was also tested.

2.4. Determination of aortic superoxide dismutase

Aortic superoxide production was determined as described previously [26]. Briefly, equilibrated segments of thoracic aorta were incubated at 37 °C in albumin-buffer (pH7.4) of the following composition (in mM): Na^+ 144.93, K^+ 7.23, Cl^- 138.77, $H_2PO_4^-$ 4.55, HPO_4^{2-} 8.03, glucose 5.5 and bovine serum albumine (0.1%, weight volume^{-1}). This buffer was enriched with lucigenin (0.5 mM) and superoxide production was calculated from chemiluminescence measurements.

2.5. Measurement of NO production

Quantitative determination of NO production was carried out as described by Zhu et al [27]. Aortic segments were loaded with the indicator by incubating them for 30 min at 37 °C in HEPES-buffered PSS (pH 7.4) containing 5 μM 4,5-diaminofluorescein. Once loading was finished, the vessels were rinsed three times and placed in a chamber containing HEPES-buffered PSS maintained at 37 °C with a water bath. L-arginine (100 μM) was added to the chamber during measurements to ensure adequate substrate availability for NO synthase.

2.6. Detection of superoxide anion

Superoxide anion (O_2^-) production by endothelium functional segments was measured as lucigenin-derived chemiluminescence in the presence of 5 μM lucigenin after stimulation with 100 μM nicotinamine adenine dinucleotide phosphate reduced form (NADPH) [28]. Each tissue sample was placed into 2 mL modified Krebs–Ringer solution, pH 7.40, and prewarmed to 37 °C for 1 h under a supply of carbogen. Immediately before measurement, rings were transferred into scintillation tubes filled with 500 μL Krebs–Hepes solution, pH 7.40, at 37 °C. Coelenterazine was added to give a final concentration of 5 μmol/L. L-NAME (1 mM) was used to inhibit the reaction of O_2^- with NO. Other NOS inhibitors interfere with NADPH-dependent reduction [28]. To estimate the true O_2^- production, the values with SOD were subtracted from those obtained in its absence.

2.7. Statistical analysis

All data were expressed as mean ± SEM. Relaxation was expressed as percentage of the active tension generated by phenylephrine. The half maximum effective concentration (EC_{50}) response to ACh was estimated by linear regression from log concentration-effect curves. Two-group mean comparisons were made using two-tailed Student's *t* test. Comparisons between every two groups were performed using one-way ANOVA. Differences were considered statistically significant at $p<0.05$.

3. Results

3.1. Effect of high glucose on relaxation of aortic rings

There were no significant differences in relaxation responses to ACh in rat aortic rings between any of the pretreated groups (data not shown). After incubation with rings for 24 h, ACh evoked a significant concentration-dependent relaxation in control group (Fig. 1 and Table 1). As shown in Fig. 1 and Table 1, the maximal relaxation (E_{max}) value was decreased ($p<0.01$), and the EC_{50} values were increased ($p<0.01$) significantly in the high glucose group (25 mM) compared with the control group. Exposure of aortic rings to high glucose for 24 h significantly attenuated relaxation responses to ACh in a dose-dependent manner compared with the control group ($p<0.05$) (Fig. 1). However, there were no significant differences between mannitol (25 mM) group and the control group in E_{max} and EC_{50} ($p>0.05$) (Fig. 1 and Table 1). The SNP-induced EIR was not different between the high glucose group and the control group ($p>0.05$) (Fig. 2).

3.2. Effects of sarpogrelate hydrochloride on the inhibition of EDR induced by high glucose

As shown in Fig. 3 and Table 1, treatment of aortic rings with sarpogrelate hydrochloride (0.1–10 μM) significantly ameliorated the impaired EDR elicited by high glucose in a dose-dependent manner. However, treatment of the rings with L-arginine (3 mM) partially prevented the high glucose-induced impairment of EDR. A similar effect was observed after treatment of aortic rings with SOD (200 U/mL, Fig. 4 and Table 1). However, in the combined presence of SOD and L-arginine, the high glucose-induced inhibition of EDR was almost completely abolished, which was very similar to the effect of sarpogrelate hydrochloride (10 μM). In addition, incubation of aortic rings in the presence of both L-NAME (10 μM) and sarpogrelate hydrochloride (10 μM) significantly abolished the protective effect of sarpogrelate hydrochloride on the impairment of EDR induced by high glucose (Fig. 5 and Table 1). The relaxation responses to ACh of aortic rings were not significantly different among the three groups (the control group, sarpogrelate group, and SOD plus L-arginine group) (Fig. 6 and Table 1). All these results suggested that both the deleterious effects of high glucose and the protective effects of sarpogrelate hydrochloride was correlated with oxidative stress and NO production/release in endothelial cells.

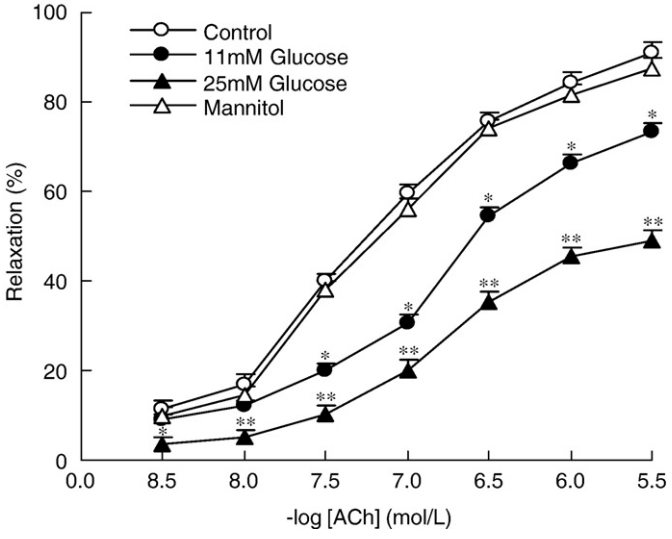

Fig. 1. Effects of high glucose on endothelium-dependent relaxation in response to acetylcholine. Values are mean ± SEM. $n=8$. *$p<0.05$, **$p<0.01$ versus the control group.