Effects of a Lipoxygenase Inhibitor, Panaxynol, on Vascular Contraction Induced by Angiotensin II

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ABSTRACT—We investigated whether a lipoxygenase inhibitor, panaxynol, affected the vascular contraction induced by angiotensin (Ang) II and the mean arterial pressure in spontaneously hypertensive rats (SHR). Panaxynol suppressed dose-dependently the contractile responses induced by 30 nM Ang II in isolated intact and endothelial cell-denuded aorta in the hamster. IC₅₀ values in the intact and endothelial cell-denuded aorta were 23 and 20 µM, respectively. In SHR, the mean arterial pressure after injection of 30 and 60 mg/kg panaxynol was reduced, and the maximum hypotensive values were 23 and 48 mmHg, respectively. Thus, lipoxygenase products may affect the renin-angiotensin system.

Keywords: Lipoxygenase, Angiotensin II, Contraction

Arachidonic acid is dominantly metabolized through the cyclooxygenase and lipoxygenase pathways. The roles of cyclooxygenase products such as thromboxane A₂ and prostaglandin I₂ in vascular reactivity have been well known, while those of lipoxygenase products such as 5-hydroxyeicosatetraenoic acid (HETE), 12-HETE and 15-HETE have not. However, previously, increase of 12-HETE, which is generated from arachidonic acid via the 12-lipoxygenase pathway, was observed in 2-kidney, one clip hypertensive (2K1C) rats and spontaneously hypertensive rats (SHR) (1, 2). Recently, we demonstrated that 12-HETE directly potentiates vascular contraction induced by angiotensin (Ang) II in isolated hamster aorta (3). These findings suggest that 12-HETE may influence the development of hypertension.

Panaxynol (9Z-heptadeca-1,9-dien-4,6-diyn-3-ol) was isolated from Ginseng radix, “Fang-Feng” (4). It has been reported that panaxynol has anti-inflammatory and anti-platelet aggregatory effects (5). Panaxynol inhibited 12-lipoxygenase of leukocyte-type and its IC₅₀ value was 1 µM (6). Although this compound had slight effects on cyclooxygenase, its IC₅₀ value was higher than 100 µM (6). Therefore, this compound can specifically inhibit the formation of lipoxygenase products such as 12-HETE. In the present study, we investigated the effect of a selective lipoxygenase inhibitor, panaxynol, on Ang II-induced vascular contraction in isolated hamster aorta and that on blood pressure in SHR.

Panaxynol was isolated from Saposhnikoviae radix as described previously (4). Eighteen male hamsters weighing 150–160 g and 12 male SHR weighing 250–300 g were purchased from Japan SLC (Shizuoka). The experimental procedures for animals were in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

The hamsters were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the aorta was isolated. The hamster aorta was cut into a helical strip, 10 mm in length and 1.0 mm in width. In some experiments, the endothelium was removed by gently rubbing the intimal surface with a cotton pellet (7). The strip was placed in an organ bath under a resting tension of 0.8 g. The bathing medium was Tyrode’s solution consisting of 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 0.42 mM NaH₂PO₄, 12 mM NaHCO₃ and 5.7 mM glucose, pH 7.4. The medium was continuously aerated with O₂/CO₂ (95:5), which was maintained at 37°C. After the strip was equilibrated, Ang II (final concentration of 30 nM) was added to the bathing medium. The medium was washed out twice for 15 min each time with fresh Tyrode’s solution and equilibrated for 30 min. The step for the Ang II
response was repeated twice, and the third Ang II response was regarded as the Ang II control response. After the response, the medium was washed out twice for 15 min each time with fresh Tyrode's solution. To study the effect of panaxynol on the Ang II-induced vascular contraction, we observed the Ang II response after pre-incubation for 30 min with 10, 30, 100 or 300 μM of panaxynol.

The SHR were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the left carotid artery and femoral vein were cannulated with polyethylene tubes (PE-50). The rats were allowed to recover for 1 day after the operation, and then the carotid arterial catheter was connected to a pressure transducer (TP-200T; Nihon Kohden, Tokyo). Panaxynol (final concentration of 10, 30 or 60 mg/kg body weight) was administered intravenously in a volume of 200 μL/kg body weight as a solution. The mean arterial pressure (MAP) was monitored continuously and was measured before and 5, 10, 30, 60 min after injection of the drug.

All results are represented as means±S.E.M. Differences were considered significant when the P values were less than 0.05 with Duncan's multiple range test.

The contractile response induced by 30 nM Ang II in isolated hamster aorta was 0.28±0.02 g, and this control response was regarded as 100%. After preincubation with panaxynol of 10, 30, 100 and 300 μM for 30 min, the contractions of the Ang II were suppressed to 92.5%, 42.8%, 18.8% and 1.2%, respectively. Panaxynol of 30, 100 and 300 μM significantly suppressed the Ang II-induced vascular contractions, and the IC₅₀ value was 23 μM (Fig. 1). In endothelial cell-denuded aorta, panaxynol also suppressed the contractions dose-dependently, with an IC₅₀ of 20 μM (Fig. 1).

Figure 2 shows the effects of panaxynol on the MAP in SHR. The MAP in SHR was 178±12 mmHg before the injection of panaxynol. After injection of 10 mg/kg panaxynol, the MAP was reduced by 2.4 and 10.0 mmHg at 5 and 10 min, respectively, while at 30 min, the MAP was recovered to the control level. The MAP after injection of 30 and 60 mg/kg panaxynol was decreased and reached at a peak at 10 min. The maximum hypotensive value after injection of 30 and 60 mg/kg panaxynol was 23 and 48 mmHg, respectively.

Recently, we demonstrated that a 12-lipoxygenase product, 12-HETE, directly potentiates the Ang II-induced contractile response in isolated hamster aorta (3). However, the norepinephrine-induced contractile response was not affected by 12-HETE (3). It is thought that different agonists of vascular contraction depend on different mechanisms. Norepinephrine-induced contraction depends on extracellular Ca²⁺ levels, while Ang II-induced contraction is sustained in a Ca²⁺-free medium.

Fig. 1. Effects of panaxynol on the Ang II-induced contractile responses in isolated hamster vessels. Open and hatched columns are the responses in intact (n=6) and endothelial cell-denuded vessels (n=6), respectively. Vertical bars represent the mean±S.E.M. *P<0.05, **P<0.01 vs each control Ang II response.

Fig. 2. Effects of intravenous administration of panaxynol (○, 10; △, 30; □, 60 mg/kg) on mean arterial pressure in SHR. Each point represents the mean±S.E.M. of data of 4 rats.
duced by Ang II and by norepinephrine, 12-HETE may potentiate vascular contraction via the increase of intracellular calcium levels by Ang II but not by norepinephrine, and the mechanism may depend on the interaction between 12-HETE and Ang II in signal transduction with the production of diacylglycerol and inositol triphosphate, but not on calcium channels. These findings suggest that inhibition of lipoxygenase may result in suppression of the contractile response induced by Ang II.

Panaxynol inhibits various lipoxygenases, and IC50 values for 5-lipoxygenase, 12-lipoxygenases of leukocyte-type and platelet-type and 15-lipoxygenase are 2, 1, 67 and 4 μM, respectively, while the IC50 value for cyclooxygenase is higher than 100 μM (6). In the present study, with an IC50 value of 23 μM, panaxynol suppressed the vascular contraction induced by Ang II in hamsters, an animal in which panaxynol can inhibit the lipoxygenase pathway but cannot influence the cyclooxygenase pathway. Furthermore, in the presence of indomethacin, the effects of panaxynol was also observed in isolated hamster aorta (data not shown). Vascular tissues dominantly contain 12-lipoxygenase and 15-lipoxygenase (10), and probably panaxynol inhibited the products via 12-lipoxygenase and 15-lipoxygenase in isolated hamster aorta. Therefore, at least, the inhibition of panaxynol on Ang II-induced vascular contraction may depend on suppression of lipoxygenase but not suppression of cyclooxygenase.

In endothelial cell-denuded vessels, panaxynol also suppressed Ang II-induced vascular contraction. Nishiyma et al. (11) reported that a 12-lipoxygenase product, 12-hydroperoxyeicosatetraenoic acid (HPETE), but not 12-HETE, directly induced a vascular contractile response. It is well known that 12-lipoxygenase converts arachidonic acid to 12-HPETE, and then 12-HPETE, which is an unstable compound, is rapidly metabolized to 12-HETE. The effects of 12-HPETE were abolished in endothelial cell-denuded vessels, and 12-HPETE may suppress endothelial-derived relaxing factors or induce endothelial contractile factor. In the present study, panaxynol may suppress 12-HPETE formation in vascular tissues. However, the effects of panaxynol was also observed in endothelial cell-denuded vessels. Therefore, the effects of panaxynol on Ang II-induced vascular contraction did not depend on suppression of 12-HPETE formation.

Ang II plays an important role in the regulation of blood pressure. In SHR, both angiotensin converting enzyme (ACE) activity and Ang II level in vascular tissues, but not renin and ACE activity in plasma, were increased (12). Moreover, ACE inhibitors can lower blood pressure in this model (13). These reports suggest that the increase of Ang II generated by ACE in vascular tissues plays a crucial role in the pathogenesis of hypertension. On the other hand, the plasma 12-HETE concentration in SHR was significantly increased compared with that in Wistar Kyoto rats (14), suggesting that up-regulated 12-HETE in the circulation may be involved in the pathogenesis of hypertension. In the hypertensive model, 12-HETE production from platelets and aortic tissues was increased significantly (15). In this study, we demonstrated that inhibition of lipoxygenase products, but not cyclooxygenase products, reduced the blood pressure in SHR.

In conclusion, we demonstrated that a selective lipoxygenase inhibitor, panaxynol, inhibited Ang II-induced vascular contraction, and lipoxygenase inhibitors may be useful as anti-hypertensive drugs.

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