Effects of the Endothelin ET_A-Receptor Antagonist FR139317 on Development of Hypertension and Cardiovascular Hypertrophy in Deoxycorticosterone Acetate-Salt Hypertensive Rats

Katsuya Fujita, Yasuo Matsumura, Yohko Miyazaki, Masanori Takaoka and Shiro Morimoto

Department of Pharmacology, Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan

Received October 23, 1995 Accepted January 24, 1996

ABSTRACT—We investigated the role of endothelin-1 (ET-1) in the development of hypertension and cardiovascular hypertrophy in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. Two weeks after the start of DOCA-salt treatment, the rats were divided into two groups and were given FR139317 [(*R*)-2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]-carbonyl]amino-4-methyl-pentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)]propionyl]amino-3-(2-pyridyl) propionic acid], a specific ET_A-receptor antagonist, or its vehicle for 2 weeks. Uninephrectomized rats without DOCA-salt treatment served as controls. Vehicle-treated DOCA-salt rats developed marked hypertension after 4 weeks. FR139317 significantly suppressed the increase in systolic blood pressure with values averaging 163 ± 8 mmHg ($P < 0.05$ vs DOCA-salt rats receiving vehicle, 195 ± 9 mmHg). Morphological studies in the rats given the vehicle showed vascular medial hypertrophy, with a significant increase in the wall area and wall-to-lumen ratio. A marked decrease in vascular wall hypertrophy was observed in the FR139317-treated DOCA-salt rats. The cardiac hypertrophy in DOCA-salt hypertensive rats was also significantly reduced by FR139317. Therefore, these results suggest that ET-1 plays an important role in the development of DOCA-salt hypertension presumably by stimulating the ET_A receptor. In addition, we found that an ET_A-receptor antagonist effectively reduced cardiovascular hypertrophy in the rats, so the cardiovascular hypertrophy noted in DOCA-salt hypertensive rats may be related to ET-1.

Keywords: Endothelin-1, Endothelin ET_A receptor, FR139317, Deoxycorticosterone acetate (DOCA)-salt hypertension, Cardiovascular hypertrophy

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide isolated from vascular endothelial cells (1). ET-1 has been implicated in the pathogenesis of hypertension, both in humans and in experimental animals (2–5). Hypertension is often accompanied by an increase in thickness of the blood vessel wall in association with hyperplasia and/or hypertrophy of vascular smooth muscle cells. Elevated blood pressure itself has been considered to play a major role in vascular hypertrophy, and local vasoactive substances in the blood vessel may relate to the pathogenesis of this process (6).

Growing evidence suggests that in addition to its vasoconstrictor properties, ET-1 has a potent proliferative effect on vascular smooth muscle cells (7–9). It stimulates DNA synthesis in cultured vascular smooth muscle cells, in a dose-dependent manner; and this effect is inhibited by BQ123, an ET_A-receptor antagonist (9). Thus, vascular ET-1 may have some role in the patho-

genesis of the vascular hypertrophy seen in the presence of hypertension.

We recently reported that vascular ET-1 content is increased in deoxycorticosterone acetate (DOCA)-salt hypertensive rats and that an intravenous bolus injection of FR139317 [(*R*)-2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]-carbonyl]amino-4-methyl-pentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)]propionyl]amino-3-(2-pyridyl) propionic acid], a specific ET_A-receptor antagonist (10, 11), produces a potent hypotensive effect in these rats, thereby indicating that the peptide contributes to the maintenance of DOCA-salt-induced hypertension (12). To further explore the role of ET-1 in the pathogenesis of DOCA-salt-induced hypertension, we examined the effect of FR139317 on the development of hypertension and vascular hypertrophy in DOCA-salt hypertensive rats.

MATERIALS AND METHODS

Experimental protocol

Male Sprague-Dawley rats (SLC, Inc., Hamamatsu), weighing 160–180 g, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the right kidney of each rat was excised through a right flank incision. After a 1-week recovery period, these rats were subcutaneously administered DOCA (15 mg/kg) suspended in corn oil, twice weekly, and 1% NaCl was added to their tap water for drinking. Control rats were uninephrectomized but not given DOCA or salt. Two weeks after the start of DOCA-salt treatment, these rats were randomly divided into two groups and were given FR139317 or the vehicle for 2 weeks. There were no significant differences in body weights and blood pressures among the two groups before the initiation of drug treatment. FR139317 (32 mg/kg) or vehicle in a volume of 1 ml/kg was given intraperitoneally twice daily. Systolic blood pressure was monitored once a week by the tail-cuff method at 3–5 hr after drug administration. Two weeks after the start of drug administration, all the rats were anesthetized, exsanguinated and the heart was excised. Cardiac hypertrophy was assessed by the left ventricular weight (including septum)-to-body weight ratio.

Morphometric analyses

The thoracic aorta and superior mesenteric artery of each rat were placed in a vial of 10%-formaldehyde neutral buffer solution for later analysis. Cross sections from the thoracic aorta and mesenteric arteries were cut 0.5- μ m-thick and stained with Elastica-van Gieson. The vessel wall area, lumen area and wall-to-lumen ratio were determined in 3–4 different cross sections of each vessel using a computerized digitizing system (IBAS II; Carl Zeiss, Frankfurt, Germany) (13).

Drugs

FR139317 was a kind gift from Fujisawa Pharmaceutical Co., Ltd., Osaka. It was dissolved in 1 N NaOH and then diluted with saline. Other chemicals were purchased from Nacalai Tesque, Inc. (Kyoto).

Statistical analyses

All values are expressed as means $\pm$ S.E.M. For statistical analyses, we used one-way analysis of variance followed by the Tukey-Kramer multiple comparisons test. Differences were considered significant at $P < 0.05$.

RESULTS

The systolic blood pressure of the DOCA-salt hypertensive and uninephrectomized rats is shown in Fig. 1.

Fig. 1. Time course in systolic blood pressure (SBP) of uninephrectomized rats (UN) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats treated with vehicle or FR139317. $\circ$, UN ($n=4$); $\bullet$, vehicle-administered DOCA-salts ($n=6$); $\blacktriangle$, FR139317-administered DOCA-salt rats ($n=5$). Values are expressed as means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$ vs UN; $\dagger P < 0.05$ vs vehicle-administered DOCA-salt.

The blood pressure of vehicle-treated DOCA-salt rats showed a time dependent increase for 4 weeks and was significantly higher than that of the uninephrectomized control rats at 3–4 weeks. DOCA-salt rats given FR139317 had a significantly smaller rise in blood pressure. Four weeks after the start of DOCA-salt treatment, the systolic blood pressure of vehicle-treated DOCA-salt rats was 195 ± 9 mmHg, whereas that of FR139317-treated DOCA-salt rats was 163 ± 8 mmHg ($P < 0.05$). At the end of the experiment (at 4 weeks), body weights of uninephrectomized control rats, DOCA-salt rats and DOCA-salt rats treated with FR139317 were 353 ± 3 , 313 ± 13 and 333 ± 9 g, respectively (differences were not statistically significant). Left ventricular weights of uninephrectomized control rats, DOCA-salt rats and DOCA-salt rats treated with FR139317 were 0.71 ± 0.01 , 0.96 ± 0.03 ($P < 0.01$ vs control rats) and 0.82 ± 0.03 g, respectively.

Figure 2 shows the left ventricular weight-to-body weight ratio of the DOCA-salt hypertensive and uninephrectomized control rats. Cardiac hypertrophy occurred in the vehicle-treated DOCA-salt hypertensive rats, with a left ventricular weight-to-body weight ratio of 3.09 ± 0.12 compared with 2.00 ± 0.04 for control rats. A significant attenuation of this hypertrophy was observed in DOCA-

Fig. 2. Left ventricular (LV) weight-to-body weight ratio of uninephrectomized rats (UN) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats treated with vehicle or FR139317. UN, $n=4$; vehicle-administered DOCA-salts, $n=6$; FR139317-administered DOCA-salt rats, $n=5$. Values are expressed as means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$ vs UN; †† $P < 0.01$ vs vehicle-administered DOCA-salt.

salt rats given FR139317, with the value being 2.48 ± 0.11 .

Figure 3 shows typical examples of light micrographs of representative cross sections of aortas (a: uninephrectomized rat, b: vehicle-treated DOCA-salt rat, c: FR139317-treated DOCA-salt rat). Typical examples of light micrographs of mesenteric arteries (a: uninephrectomized rat, b: vehicle-treated DOCA-salt rat, c: FR139317-treated DOCA-salt rat) are shown in Fig. 4. Increase in vascular medial thickness, a characteristic finding for hypertensive arterial hypertrophy, was clearly evident in the vehicle-treated DOCA-salt rats. The vehicle-treated DOCA-salt rats showed a substantial increase in wall area of the thoracic aorta (46%) and mesenteric artery (83%), as summarized in Table 1. Similarly, the wall-to-lumen ratio of vehicle-treated DOCA-

salt rats was significantly greater than that of the uninephrectomized rats. FR139317 markedly inhibited the vascular wall hypertrophy in both blood vessels. The vascular wall area of FR139317-treated DOCA-salt rats was not significantly different from that of uninephrectomized controls.

DISCUSSION

To examine the role of ET-1 in the development of DOCA-salt hypertension, we observed the effects of FR139317, a specific ET_A -receptor antagonist, on the development of DOCA-salt hypertension. FR139317 suppressed the development of DOCA-salt-induced hypertension in the rats. Morphological studies in DOCA-salt rats showed arteriosclerotic changes, with a significant increase in the wall area and wall-to-lumen ratio. A marked decrease in the vascular wall hypertrophy was observed in FR139317-treated DOCA-salt rats. Together with the results of our previous study (12), it seems likely that ET-1 has an important role in the development and maintenance of DOCA-salt hypertension.

Several groups of investigators reported the effect of ET-receptor antagonists on blood pressure in experimental hypertensive animals. BQ123, a specific ET_A -receptor antagonist, significantly reduced arterial pressure in stroke-prone spontaneously hypertensive rats but not in stroke-resistant spontaneously hypertensive rats (14). FR139317 had no effect on the blood pressure of spontaneously hypertensive rats and that of 2 kidney 1-clip renal hypertensive rats (10). On the other hand, there is good evidence that acute treatment with an ET_A -receptor antagonist has a potent hypotensive effect in established DOCA-salt hypertensive rats (12, 15–17). Furthermore, the result of our present study showed that two weeks of treatment with FR139317, an ET_A -receptor antagonist, suppressed the development of DOCA-salt hypertension, suggesting that ET-1 and activation of ET_A receptor may be a pathogenic factor in DOCA-salt hypertension.

The mechanism by which ET-1 may promote DOCA-salt hypertension is not clear, but some factors are worthy

Table 1. Morphological analysis of the thoracic aorta and mesenteric artery

Group	n	Aorta		Mesenteric artery	
		wall area (mm ²)	wall-to-lumen ratio	wall area (mm ²)	wall-to-lumen ratio
UN	4	0.41 ± 0.01	0.27 ± 0.01	0.12 ± 0.01	0.33 ± 0.02
DOCA-salt	6	0.63 ± 0.03^b	0.36 ± 0.01^b	0.22 ± 0.01^b	0.48 ± 0.03^a
DOCA-salt + FR139317	5	0.50 ± 0.02^c	0.28 ± 0.01^c	0.15 ± 0.01^c	0.43 ± 0.03

n indicates the number of rats in each group. Values are expressed as means $\pm$ S.E.M. UN: uninephrectomized rats, DOCA: deoxycorticosterone acetate. ^a $P < 0.05$, ^b $P < 0.01$ vs UN; ^c $P < 0.01$ vs DOCA-salt.

Fig. 3. Photomicrographs show the morphology of thoracic aortas. a: uninephrectomized rats (control), b: vehicle-administered deoxycorticosterone acetate (DOCA)-salt hypertensive rats, c: FR139317-administered DOCA-salt hypertensive rats.

Fig. 4. Photomicrographs show the morphology of mesenteric arteries. a: uninephrectomized rats (control), b: vehicle-administered deoxycorticosterone acetate (DOCA)-salt hypertensive rats, c: FR139317-administered DOCA-salt hypertensive rats.

of consideration. It is well known that DOCA-salt hypertension is characterized by the over-expression of extracellular fluid volume and an initial increase in cardiac output. However, change in cardiac output is not required in the development of DOCA-salt hypertension (18). Therefore, changes in peripheral vascular resistance may be of primary importance in the generation of DOCA-salt hypertension. We and others demonstrated that vascular ET-1 concentration was significantly increased in DOCA-salt hypertensive rats, compared with findings in age-matched control rats (12, 19). In addition, we noted that vascular ET-1 concentrations are closely related to systolic blood pressure in the case of DOCA-salt hypertensive rats (12). Since ET-1 is a potent vasoconstrictor peptide, increased vascular ET-1 with its associated sustained-vasoconstriction may be involved in the increase in systemic vascular resistance in DOCA-salt hypertension. ET-1 not only exerts direct vasoconstrictor effects but also potentiates contractile responses to other vasoconstrictor substances such as norepinephrine (20, 21). The potentiative effects induced by ET-1 occurred with even a threshold concentration of the peptide. Since it has been suggested that sympathetic tone is increased in DOCA-salt hypertensive rats (22), this indirect effect of ET-1 may be more important for the increase in systemic vascular resistance in DOCA-salt hypertension. More recently, we noted that ET-1 and ET_A receptor might be related to the renal hemodynamic abnormality in DOCA-salt hypertensive rats (23). Since renal function is considered to play the central role in the long-term control of blood pressure and the pathogenesis of hypertension, the abnormality in renal hemodynamics may participate in the development of hypertension.

Hypertension, in humans and in experimental animals, is often accompanied by vascular hypertrophy (24). In our study, morphological analysis of aortae and mesenteric arteries in DOCA-salt rats showed arteriosclerotic changes, with a significant increase in the wall area and wall-to-lumen ratio. Elevated blood pressure itself has been considered to play a major role in the vascular hypertrophy. It was reported that these structural changes are not simply a response to elevated blood pressure. ET-1 has potent mitogenic and hypertrophic properties (7–9). Both the proliferative and the contractile actions of ET-1 are mediated by the ET_A receptor in vascular smooth muscle cells (9). Thus an increased vascular ET-1 may play a role in vascular hypertrophy in DOCA-salt hypertensive rats. In the present study, a marked decrease in vascular wall hypertrophy was observed in FR139317-treated DOCA-salt rats. The decrease in vascular wall hypertrophy of aortae and mesenteric arteries was clear-cut, even though the systolic blood pressure after treatment with FR139317 remained at the hypertensive level.

The wall area was not significantly different from that of uninephrectomized controls. The cardiac hypertrophy in DOCA-salt hypertensive rat has been documented (25–27). Several comparative studies revealed a dissociation between the antihypertensive effect of drugs and the regression of cardiac hypertrophy. Lee et al. (27) showed that effective antihypertensive therapy with a calcium antagonist did not prevent cardiac hypertrophy in DOCA-salt hypertensive rats. It has also been reported that, for the same reduction in hypertension, there is a proportional regression of cardiac hypertrophy with the β -blocker propranolol, but no change with the α_2 -agonist rilmenidine (28). Thus, the cardiac hypertrophy induced by DOCA-salt treatment may be due to another effect of DOCA-salt treatment, independent of blood pressure elevation. In the present study, the left ventricular weight-to-body weight ratio was increased in DOCA-salt hypertensive rats, and the hypertrophy was significantly reduced in FR139317-treated DOCA-salt rats. ET-1 is produced in vascular endothelial cells and cardiac myocytes (29, 30). Other investigators have shown that ET-1 is a potent growth factor for cardiomyocytes (31, 32). Furthermore, cardiac ET-1 production is increased in pressure or volume overload cardiac hypertrophy in vivo (33, 34). Taken together, our results suggest that ET-1 may be one possible candidate for the factor that induces the cardiovascular hypertrophy seen in DOCA-salt hypertensive rats. However, the possibility that the suppression of the cardiovascular hypertrophy by FR139317 is in part due to the agent-induced hypotension cannot be ruled out. Further studies are required to determine the relative role of the ET_A receptor in the cardiovascular hypertrophy in DOCA-salt hypertensive rats.

In a recent study, Li et al. (35) reported that bosentan, a nonselective ET-receptor antagonist, suppressed the elevation of blood pressure and vascular hypertrophy in DOCA-salt hypertensive rats. They suggested a pathophysiological role for ET-1 in DOCA-salt hypertensive rats. Our present results provide support for this notion. However, it is difficult to evaluate the important ET receptor subtype from their study. Since FR139317 is a highly selective antagonist for ET_A receptors, one may conclude that such a receptor subtype is primarily involved in the pathogenesis in DOCA-salt hypertension. Given the fact that the presence of the ET_B receptor in vascular smooth muscle cells has been demonstrated (36, 37), their potential role cannot be excluded. In contrast to our study using FR139317, Li et al. noted that bosentan did not alter the ratio of heart weight to body weight in DOCA-salt hypertensive rats, despite the blood pressure lowering effect of the drug. The reason for this discrepancy is unclear, but it is related to the difference in these antagonist-induced hypotensive potencies. Determi-

nation of the precise mechanisms involved in the different effects of these two drugs on the cardiac hypertrophy requires detailed study.

In conclusion, the results of our study indicate that ET-1, most likely through the stimulation of the ET_A receptor, plays an important role in the development of DOCA-salt hypertension. In addition, an ET_A-receptor antagonist effectively reduced cardiovascular hypertrophy, suggesting that the cardiovascular hypertrophy noted in DOCA-salt hypertensive rats may be related to ET-1.

Acknowledgments

This study was supported in part by the Science Research Promotion Fund of the Japan Private School Promotion Foundation. We thank Dr. M. Furuya, Suntory Institute for Biomedical Research, for assistance with morphometric studies and M. Ohara for critical comments.

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