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Comparison of the relative importance of vascular and plasma drug concentrations to the hypotensive effect of telmisartan in rats

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Abstract

Introduction: To clarify the importance of the vascular concentration of an angiotensin II receptor blocker (ARB) to its hypotensive effect, the relationships between the drug concentrations in plasma and vascular tissues and the hypotensive effect after administration of an ARB were compared.

Materials and methods: In spontaneously hypertensive/NDmcr-cp rats (SHR/NDmcr-cp), systolic blood pressure (SBP) and angiotensin II-induced vascular contraction were measured 2 h and 24 h after administration of telmisartan (3 mg/kg). Plasma and vascular concentrations of telmisartan were also measured at 2 h and 24 h.

Results: SBP was significantly lower 2 h after administration of telmisartan, and the significant hypotensive effect was continued until 24 h. A significant attenuation of angiotensin II-induced vascular contraction at 2 h was also continued until 24 h. No significant difference between 2 h and 24 h was observed both in SBP and angiotensin II-induced vascular contraction. Vascular concentration at 24 h was 90.0% when the concentration at 2 h was assumed to be 100%, and no significant difference was observed. However, the plasma concentration of telmisartan at 2 h was significantly decreased by 88.2% at 24 h.

Conclusion: The vascular drug concentration, not the plasma drug concentration, may be related to the hypotensive effect after administration of telmisartan.

Keywords

Angiotensin II receptor blocker, plasma, vascular tissues, drug concentration, lipophilicity

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Introduction

Angiotensin II plays an important role in regulating blood pressure by stimulating angiotensin II receptors, and its stimulation also augments oxidative stress, resulting in vascular dysfunction. Therefore, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) may be more useful for preventing vascular dysfunction than other hypotensive drugs. However, a more important effect for patients with hypertension has been shown to be the production of a lower blood pressure that is stable for 24 h after administration of medication in the clinic.[1,2]

In general, drug efficacy is thought to be related to plasma drug concentration. However, the hypotensive effect of ACE inhibitors may be dependent on the vascular ACE inhibitory effect rather than on the plasma effect.[3,4] A significant correlation between vascular ACE activity and systolic blood pressure (SBP) was observed after administration of ACE inhibitors in spontaneously hypertensive (SHR), but no correlation was seen between plasma ACE activity and SBP.[4] However, the importance of vascular ARB concentration on

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the hypotensive effect has been unclear, because measurement of drug concentrations in vascular tissues has been difficult. Recently, we succeeded in measuring the concentration of ARB in plasma and vascular tissues using a novel method, matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) and imaging mass spectrometry (IMS) in the selected reaction monitoring (SRM) mode.[5] In the present study, to clarify the importance of the vascular concentration of an ARB on the hypotensive effect, the hypotensive effect, angiotensin II-induced vascular contraction, and drug concentrations in plasma and vascular tissue after administration were measured.

Materials and methods

Animals

Ten-week-old male spontaneously hypertensive/NDmcr-cp rats (SHR/NDmcr-cp) were obtained from Japan SLC Inc. (Shizuoka, Japan). All procedures complied with the guidelines on animal care of the local Ethics Committee on the Use of Animals (protocol number 8815, Shiga Research Center, Nissei BILIS, Koka, Japan).

Each rat was orally administered 3 mg/kg of telmisartan. SBP was monitored by tail-cuff plethysmography (BP-2000, Visitech Systems, Apex, NC, USA) 2 h and 24 h after oral drug administration, and the rats were then anesthetized with isoflurane to obtain blood and vascular tissues.

Vascular responses in the isolated artery

Isolated rat carotid arteries were cut into 10 mm × 1.0 mm helical strips and placed on a myograph under a resting tension of 1.0 g in Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 12 mM $NaHCO_3$, and 5.7 mM glucose, pH 7.4) at 37°C and continuously bubbled with 5% CO_2 in O_2. The strips were initially vasoconstricted with 50 mM KCl, and the response of each strip served as the reference for angiotensin II (30 μM)-induced contraction of the corresponding strip.

MALDI-TOFMS and MALDI-IMS analyses

To measure plasma drug concentration, a MALDI matrix consisting of 7 mg/mL of α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich Japan, Tokyo, Japan) was directly mixed with plasma, and the mixture was placed on a MALDI plate (Bruker Daltonics, Billerica, MA, USA).[5]

To measure vascular drug concentration, aortic tissue was frozen in TissueTech™ (Sakura Fineteck, Osaka), and each block was sectioned at a thickness of 12 μm.[6] The sections were mounted on indium-tin-oxide-coated glass slides (Bruker Daltonics), and then α-CHCA matrix was coated onto the sections.

The concentration of telmisartan in plasma and vascular slices was quantified using MALDI-TOFMS and -IMS in the SRM mode on an Autoflex Speed TOFMS (Bruker Daltonics) equipped with a Smartbeam™-II solid-state laser (wavelength, 355 nm; focused diameter, 20 μm; repetition rate, 500 Hz).[5] Telmisartan detected by MALDI-TOFMS and -IMS in the SRM mode is all active form, and its metabolites did not be detected.

With respect to the precision of measuring telmisartan in plasma ($n = 8$), the variability of results was 5.8%, and the accuracy values ranged between from −11.0% to −3.7%. The limit of telmisartan measurement in plasma by MALDI-TOFMS in the SRM mode was 2.0 nM. The precision for the measurement of telmisartan in sliced aorta was evaluated using dropped telmisartan on a glass slide ($n = 8$). The variability of results was 4.6%, and the accuracy values ranged from −2.3% to 9.2%. The limit of telmisartan measurement in sliced aorta by MALDI-IMS in the SRM mode was 0.2 pmol/mm^2.

Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). The significance of differences between mean values for multiple groups was evaluated using one-way analysis of variance followed by Scheffe's test. Differences were considered significant at a p value < 0.05.

Results

SBP and angiotensin II-induced vascular contraction

SBP was significantly lower at 2 h than at pre-treatment, and a significant lowering effect was also observed at 24 h (Figure 1(a)). There was no significant difference between 2 h and 24 h (Figure 1(a)).

Angiotensin II-induced vascular contractions were attenuated by 94.6% and 90.6% at 2 h and 24 h, respectively, when the contraction before administration of telmisartan was assumed to be 100% (Figure 1(b)). No significant difference was also observed between 2 h and 24 h (Figure 1(b)).

Plasma and vascular concentrations of telmisartan

Plasma concentrations of telmisartan were 0.34 ± 0.04 μM and 0.04 ± 0.01 μM 2 h and 24 h after oral administration, respectively, and the difference was significant (Figure 2(a)).

Vascular concentrations of telmisartan were 2.82 ± 0.39 pmol/mm^2 and 2.54 ± 0.33 pmol/mm^2 at 2 h and 24 h after oral administration, respectively (Figure 2(b)). Although the vascular concentration of telmisartan tended to be lower from 2 h to 24 h, no significant difference was observed.

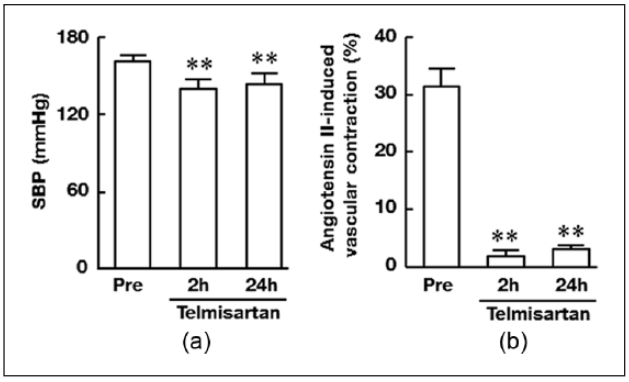

Figure 1. SBP before (pre) and 2 h and 24 h after administration of telmisartan (a). Angiotensin II-induced vascular contraction in isolated carotid arteries before (pre) and 2 h and 24 h after administration of telmisartan (b). $^{**}p < 0.01$ vs. before administration. Values are the means ± SEM of 4 rats.

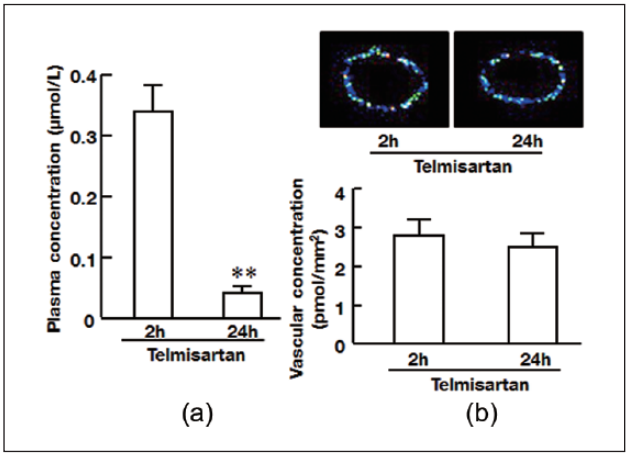

Figure 2. Plasma concentrations of telmisartan 2 h and 24 h after administration (a). Vascular concentrations of telmisartan 2 h and 24 h after administration (b). $^{**}p < 0.01$ vs. 2 h after administration. Values are the means ± SEM of 4 rats.

Discussion

In general, higher lipophilic drugs including ACE inhibitors may be more likely to remain in tissues for the long-term.[3,4] Both vascular ACE inhibition and a hypotensive effect were observed until several days after administration of a high-lipophilic ACE inhibitor (trandolapril).[3] Significant vascular ACE inhibition continued until 24 h after administration in SHR treated with the high-lipophilic ACE inhibitors trandolapril and perindopril, but not with the low-lipophilic ACE inhibitors enalapril and temocapril, although these ACE inhibitors inhibited vascular ACE activity equally 3 h after administration.[4] Like ACE inhibitors, ARBs also shows various lipophilicities. The lipophilicity index is defined as the ratio of octanol to buffer, and the values of log *p* are: −2.45 with EXP 3174 (an active metabolite of losartan); −0.96 with candesartan; −0.95 with valsartan; +1.48 with irbesartan; and +3.20 with telmisartan.[6] Among ARBs, telmisartan has been well known to show a high-lipophilic character, and telmisartan was used to clarify the importance of the vascular concentration of ARBs on the hypotensive effect.

In the present study, telmisartan showed a significant hypotensive effect 2 h after administration, and the hypotensive effect was observed until 24 h. The significant attenuation of angiotensin II-induced vascular contraction seen at 2 h after administration was also observed until 24 h. These findings suggest that blockade of angiotensin II receptors was continued until 24 h after administration, and the continuous vascular blockade resulted in lower SBP. In fact, 90% of the vascular telmisartan concentration detected 2 h after administration was maintained until 24 h. On the other hand, plasma telmisartan concentration was decreased by 87.4% from 2 h to 24 h. Thus, continuous attenuations of SBP and angiotensin II-induced vascular contraction until 24 h after administration may be dependent on the maintenance of vascular telmisartan concentration, but not plasma telmisartan concentration.

As a limitation, the data shown in Figure 2(b) may include the non-specific bound drug to tissue; the ratio of drug bound specifically to angiotensin II receptors to that bound non-specifically in vascular tissues is not known. Although different ratios of specific to non-specific binding are observed among different drugs, the tissue drug concentration is thought to reflect the pharmacological effect using the same drug. In the present study, the high maintenance of vascular drug concentration at 24 h compared with that at 2 h may reflect the long-term pharmacological effect.

In the clinical setting, it is very difficult to use vascular drug concentration as a biomarker for the hypotensive effect of ARBs. However, the importance of vascular drug concentration demonstrated in the present study may help us understand the mechanism of the hypotensive effect of ARBs and to develop long-acting pharmacotherapy. Drugs need to maintain a stable hypotensive effect for 24 h after their administration in the clinic, and evaluation of vascular drug concentration may be useful for the development of long-acting antihypertensive drugs, including ARBs.

Conclusions

In general, plasma drug concentration has been thought to be important for drug efficacy, but vascular drug concentration may be more important in reflecting the hypotensive effect when using high-lipophilic drugs such as telmisartan.

Declaration of Conflicting Interests

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