Evaluation for Antioxidant and Renoprotective Activity of Olmesartan Using Nephrectomy Rats

Daisuke Kadowaki,^a Makoto Anraku,^b Yuka Tasaki,^c Kazuaki Taguchi,^c Kazuki Shimoishi,^c Hakaru Seo,^c Sumio Hirata,^a Toru Maruyama,^a and Masaki Otagiri^a,b,c

^a Graduate School of Pharmaceutical Sciences, Kumamoto University; 5—1 Oe-honmachi, Kumamoto 862–0973, Japan; ^b Faculty of Pharmaceutical Sciences, Fukuyama University; 1 Gakuen-cho, Fukuyama, Hiroshima 729–0292, Japan; and ^c Faculty of Pharmaceutical Sciences, Sojo University; 4–22–1 Ikeda, Kamamoto 860–0082, Japan.

Received June 30, 2009; accepted September 17, 2009; published online September 25, 2009

Angiotensin II type 1 receptor blockers, which inhibit the rennin-angiotensin system, are used in the treatment of hypertension. In addition to their ability to lower blood pressure, these compounds have also been reported to protect organs, such as kidney and heart. Although the mechanisms of these protective effects are not fully understood, it is generally thought that their antioxidant effects likely play a role. The aim of the present study was to characterize the relationship between the antioxidant activity of olmesartan and its pharmacological actions such as renoprotective or blood pressure lowering effects, using 5/6 nephrectomy rats. In 5/6 nephrectomy rats, the potential antioxidant power, the ratio of oxidized to unoxidized albumin, as a marker of protein oxidation in blood, both systolic and diastolic blood pressure, plasma creatinine concentration, and amounts of protein excreted into the urine were significantly higher than the corresponding values for sham operated rats. However, olmesartan significantly suppressed these parameters within 8 weeks after oral administration in 5/6 nephrectomy rats. The oxidized albumin ratio was significantly decreased 4 weeks after the administration of olmesartan and these lower levels were maintained at 8 weeks. Furthermore, olmesartan improved radical scavenging activity of isolated albumin from rat plasma. Interestingly, a good correlation was found between the oxidized albumin ratios and renal function, whereas no correlation was found in the case of blood pressure. Based on these findings, we conclude that the antioxidant properties of olmesartan may be related to its renoprotective action rather than an antihypertensive effect.

Key words olmesartan; angiotensin II type 1 receptor blocker; antioxidant; hypertension; renoprotective effect

The renin-angiotensin system (RAS) plays an important role in regulating blood pressure (BP). It has recently been revealed that the elevated levels of RAS are associated with various organ impairments. Thus, an angiotensin II receptor blocker (ARB) and an angiotensin converting enzyme inhibitor (ACEI) are effective for treating hypertension and show protective effects for the heart or kidney. For example, in a clinical trial in which the effect of ARB valsartan and calcium channel blocker (CCB) amlodipine were compared in patients with diabetes, valsartan was found to decrease the urinary albumin excretion rate to a greater extent than CCB, while BP was reduced to the same level.11 Consequently, RAS antagonists may exert a renal protective effect that is independent of its antihypertensive effect, and may be involved in reducing of the levels of oxidative stress. In fact, contrary to CCB, ARB improved endothelium-dependent coronary dilation in hypertensive patients independent of BP reduction. These beneficial effects on coronary vasomotion might be a result of the antioxidant properties of ARBs.22

Olmesartan medoxomil, a prodrug that is converted into the active metabolite olmesartan has recently been developed in association with ARB3,4 Miyata et al. reported that the antioxidant effects of olmesartan were greater than any other ARBs in an in vitro study.5 We recently reported, in an in vitro study, that clinical concentrations of olmesartan exert antioxidant properties. This was further confirmed in a clinical trial for hemodialysis (HD) patients as well.6 We also reported that the antioxidant effects of olmesartan might be independent of its BP lowering ability. To develop a better understanding of the antioxidant effect of olmesartan in vivo, relationships between the antioxidant activity of olmesartan and pharmacological effects, such as renoprotective or BP lowering effects, were examined using the 5/6 nephrectomy rat.

MATERIALS AND METHODS

Materials Olmesartan was obtained from the Daiichi-Sankyo Pharmaceutical Co., Ltd. (Tokyo, Japan). All other chemicals were of the highest grade available from commercial sources.

Animals and Treatment Male Wister rats were purchased from Seac Yoshitomi, Ltd. (Fukuoka, Japan). The experimental protocol was inspected and approved by the Animal Care and Use Committee of School of Medicine, Kumamoto University and the law and notification of the Japanese government prior to commencement of the study. Five-week-old male rats weighing 140—150 g were subjected to a 5/6 nephrectomy, as previously described.7,8 Six weeks after the operation, the treated rats showed increased plasma concentrations of creatinine (>1.0 mg/dl), and reduced levels of creatinine clearance (1.12 approximately 1.24 ml/min). Age-matched Sham-operated rats were used for comparison. These rats were divided into three groups as follows: (a) untreated nephrectomized group (n=4—6). (b) Olmesartan (10 mg/kg/d) treated nephrectomized group (n=4—6). (c) Sham-operated rats (n=4—6). All of the rats received standard rat chow.

Effects of Olmesartan on Renal Function in 5/6 Nephrectomy Rat Olmesartan was suspended in 0.5% of carboxymethylcellulose, and the suspension was administered orally once per day at a dose of 10 mg/kg/d through a
stomach tube. Blood and urine sample were collected under diethylether anesthesia at 0, 4, 8 weeks after the administration of olmesartan. Systolic BP and diastolic BP were measured every 4 weeks in conscious rats by the indirect tailcuff method (BP-98A; Softron, Tokyo, Japan). Plasma creatinine and urinary protein were measured according to previously described methods.9)

Analysis of Oxidized Albumin Ratio by HPLC High-performance liquid chromatography (HPLC) was used to analyze serum albumin, as described previously.6,8,9) Serum samples were immediately frozen and stored at −80°C until used for analysis. Typically, 5 µl aliquots of serum were analyzed on a Shodex Asahipak ES-502N column (Showa Denko Co., Ltd., Tokyo, Japan). From the HPLC profiles of rat serum albumin (RSA), the ratios of oxidized to unoxidized albumin (oxidized albumin ratio) were estimated by dividing the oxidized form (rat non-mercaptalbumin (RNA)) by the area of the reduced form (rat mercaptalbumin (RMA)).8)

Potential Antioxidant Assay in Plasma The evaluation of the potential antioxidant in plasma samples was determined by using the ‘PAO’ test kit (Japan Institute for the Control of Aging (JaICA), Nikken SEIL Co., Ltd., Shizuoka, Japan).10,11) This assay evaluated Cu2+ levels derived by reduction of Cu2+ by the action of antioxidants present in the sample. The stable complex between Cu2+ and bathocuproine was assayed at 490 nm, with sensitivity of 22 µmol/l of reducing power. The assay was found to be linear from 1 to 2000 µmol/l of uric acid (r=0.99, p<0.01). Both within-run and between-run assay variability, tested by repeatedly assaying five samples, was always lower than 5%.

Isolated Albumin from Rat Plasma RSA samples were isolated by polyethylene glycol fractionation of blood plasma followed by chromatography on a blue Sepharose CL-6B column (Amersham-Pharmacia, Uppsala, Sweden).12,13) The samples were then dialyzed against deionized water for 48 h at 4°C, followed by lyophilization. The purity of the HSA samples was at least 95%.

Scavenging Activity of Isolated Albumin on DPPH Radicals Radical scavenging activities of isolated albumin were tested in ethanolic solution (10 ml of ethanol, 10 ml of 50 mM 2-(N-morpholino) ethane-sulfonic acid (MES) buffer (pH 5.5) and 5 ml of 0.5 mM 1,10-diphenyl-2-picrylhydrazyl (DPPH)). Radical scavenging was estimated from the decrease in absorbance of DPPH radicals at 517 nm.11,14)

Statistics Statistical significance was evaluated by ANOVA followed by the Newman–Keuls test for comparison among >2 mean values. For all analyses, p<0.05 was regarded as being statistically significant. The results are reported as the mean±S.E.

RESULTS

Effects of Olmesartan on Renal Function Chronic renal failure (CRF) rats were constructed by 5/6 nephrectomization, using a common procedure. Six weeks after the operation, blood samples were collected at 4 week intervals after the administration of olmesartan. Figure 1 shows changes in plasma creatinine levels and the amounts of urinary protein at 0, 4, 8 weeks after administration of the drug. As expected, the level of plasma creatinine and urinary protein were transitionally elevated in 5/6 nephrectomy rats, but these changes were significantly suppressed by the olmesartan treatment, indicating that olmesartan exhibits a renal protective action under these experimental conditions.

Effects of Olmesartan on Oxidized Albumin Ratio Figure 2 shows data for the ratio of oxidized albumin in rat plasma at 0, 4 and 8 weeks. No significant differences were found in oxidized albumin ratio in control rats at 0, 4 or 8 weeks. In contrast, the oxidized albumin ratio for CRF rats was significantly higher than that of control rats at 0, 4 weeks and this was maintained for entire observation period. Interestingly the administration of olmesartan led to a dramatic reduction in the oxidized albumin ratio at 4 weeks, and that this effect was also maintained for 8 weeks, indicating that
olmesartan clearly possesses antioxidant effects in vivo.

Effects of Olmesartan on BP in CRF Rat

Figure 3 shows the changes in BP of CRF rats before and at 8 weeks after olmesartan treatment. In CRF rats, both the systolic and diastolic BP were significantly higher than these of control rats. However, these values were significantly decreased by olmesartan treatment.

Effects of Olmesartan on Plasma Antioxidant Defense System in CRF Rat

Since the potential antioxidant (PAO) in plasma was estimated by the reducing power of Cu, it directly evaluated the capacity of antioxidant defense system in plasma. Figure 4A shows the effect of olmesartan on PAO in CRF rats. PAO remarkably reduced in CRF rats, but it was significantly recovered by olmesartan treatment for 8 weeks. To further evaluate the antioxidant property of olmesartan directly, albumin was isolated from plasma of CRF rats and its radical scavenging activity against DPPH radical was evaluated. As shown in Fig. 4B, DPPH radical scavenging activity of isolated albumin was decreased in CRF rats, but it was significantly ameliorated by the administration of olmesartan for 8 weeks. These results indicated that olmesartan markedly improved antioxidant defense ability in plasma of CRF rats.

Relationship between Pharmacologic and Antioxidant Effect of Olmesartan

To characterize the relationship between antioxidant activity and the pharmacologic effects of olmesartan, such as antihypertensive and renoprotective effects, we examined the correlation between the oxidized albumin ratio and plasma creatinine levels or urinary protein excretion at 8 weeks after the administration of olmesartan. Interestingly, a good correlation was found between the oxidized albumin ratio and both plasma creatinine levels and urinary protein (Fig. 5), whereas no relation was found in the case of BP (Fig. 6).

DISCUSSION

In CRF, the balance between pro-oxidants and antioxidants is shifted toward an increased oxidative stress. In addition, chronic kidney disease (CKD) patients present a deficiency in different components of antioxidant defense mechanisms, including reduced levels of vitamins C. Therefore, it has been proposed that increased oxidative stress is a non-traditional factor for both the progression of renal damage and the development of complications. Consequently, many studies have been carried out, in attempts to characterize the antioxidant properties of CKD medicines including ARBs.

We previously reported that olmesartan exhibited antioxidant activity in HD patients, even though it was used as a clinical dose for the first time. From the findings of this clinical study, it is also possible that the antioxidant properties of olmesartan are independent of its antihypertensive activity. Therefore, we examined the issue of whether or not the antioxidant properties of olmesartan are related to its phar-
macological activity, such as blood lowering effect and renoprotective action using CRF model rats.

Using the ratio of oxidized albumin as a marker for oxidative stress in the blood circulation, the antioxidant activity of olmesartan was examined in CRF rats and this was achieved earlier than its antihypertensive effect. These phenomena were very similar to the results obtained from a clinical study involving HD patients. For example, in both CRF rats and HD patients, the oxidized albumin ratio was significantly decreased at 4 weeks by the administration of olmesartan (Fig. 2). Moreover the reduction in BP by olmesartan at 8 weeks was also similar to the HD conditions (Fig. 3). These similarities lead us to conclude that olmesartan acts as an antioxidant in the early phase of CRF in vivo as well as in HD patients. To further assess the antioxidant effect of olmesartan in blood circulation, PAO activity and DPPH radical scavenging activity of albumin isolated from rat plasma were determined before and after olmesartan treatment in CRF rats. As shown in Fig. 4, olmesartan significantly recovered these parameters, indicating the improvement of antioxidant defense ability in plasma of CRF rats.

Interestingly, changes in the oxidized albumin ratio were significantly correlated with plasma creatinine levels and urinary protein excretion, while no correlation was found in the case of changes in BP. This supports the view that the augmentation of oxidative stress may be involved in the progression of renal failure in vivo, and that the antioxidant properties of olmesartan play an important role in renal protection rather than BP lowering effects. It has recently been reported that protein urea levels might be one of the risk factors for cardiovascular events as well as for the onset of renal disease.20,21 Thus, it would be expected that the reduction in oxidative stress by olmesartan did not only lead to protection of the kidney but other organs as well, such as the vascular system, heart and brain. In fact, losartan, one of the ARBs, showed preventive effects for cerebral vascular events in a LIFE study, and many other studies, such as the Jikei Heart study or the ONTARGET study report that ARBs have the potential to reduce death resulting from cardiovascular events.22–25

It is well established the antihypertensive effect of olmesartan is mediated by the inhibition of angiotensin II type 1 receptor (AT1R) activity.26,27 In addition, AT1R activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, leading to the subsequent production of reactive oxygen species. Therefore, we initially expected that the antioxidant effect of olmesartan might be caused by the inhibition of NADPH oxidase via AT1R binding.28,29 However, our previous clinical data and the present findings suggest that the antioxidant effect of ARBs is likely to be independent of antihypertensive effects.6 Therefore, we hypothesize that the mechanism of antioxidant property of olmesartan is not only due to the inhibition of NADPH oxidase activity mediated by AT1R but that other mechanisms may also be involved. In fact, several recently reported findings support this conclusion. For example, we previously reported on the radical scavenging activity of olmesartan based on the inhibition of the generation of albumin hydroperoxides via γ-irradiated hydroxyl radicals and the auto oxidation of albumin.60 Moreover, Miyata et al. reported that olmesartan suppresses the Fenton reaction by chelating metals and the production of carbon center radicals or hydroxyl radicals.61 Very recently, Naya et al. reported that olmesartan exerts the antioxidant effects by inducing SOD activity in blood vessels, and this effect might be related to the protection of vascular endothelial cells.23

Recently, it has been proved that ARBs can be classified into two groups depending upon inverse agonist activity to AT1R.26,27 Since olmesartan possesses strong inverse agonist action to AT1R among ARBs, the issue of whether inverse agonist ability affects the antioxidant activity of ARBs constitutes a subject of interest. In addition, olmesartan is typically not administrated alone, but is also frequently combined with CCB or diuretics in clinical settings. Consequently, combinational preparations with those anti-hypertensive drugs have been developed and are widely used in CKD patients. Therefore, it will be necessary to clarify whether other anti-hypertensive drugs affect the antioxidant activity of olmesartan and to compare the antioxidant activity of olmesartan with other ARBs. Studies are currently underway in our laboratory to address these issues.

In conclusion, the findings presented herein demonstrate that olmesartan possesses antioxidant effects which might be independent upon its antihypertensive effects. Since oxidative stress is significantly associated with the progression of CKD and the development of complications such as cardiovascular disease, antioxidant activity beyond the lowering of BP by olmesartan could be involved in its organ protection action in CKD patients.

REFERENCES