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Effects of olmesartan medoxomil as an angiotensin II-receptor blocker in chronic hypoxic rats

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Abstract

We established a rat chronic alveolar hypoxia in vivo model to evaluate the efficacy against hypoxic pulmonary hypertension of a new angiotensin II-receptor I blocker, olmesartan medoxomil. Three groups of rats were established: rats exposed for 2–6 weeks to 10% oxygen atmosphere in a normobaric chamber; hypoxic rats treated with olmesartan medoxomil oral administration (5 mg/day) every day; and control rats fed in a normoxic condition. After hypoxia treatment, the presence, etiology and severity of pulmonary hypertension, was echocardiographically evaluated, and expressions of brain natriuretic peptide (BNP), transforming growth factor (TGF-β) and endothelin-1 genes measured by both immunohistochemical assay and real-time polymerase chain reaction. Olmesartan medoxomil significantly reduced the induction of hypoxic cor pulmonale not only on echocardiographical observations but also in BNP, TGF-β and endothelin gene expressions in molecular studies. However, systolic blood pressure was independent of olmesartan medoxomil. The present study clearly indicates that the angiotensin II-type I-receptor blocker olmesartan medoxomil has significant efficacy for hypoxic cor pulmonale.

Keywords: Hypoxic cor pulmonale; Angiotensin II-receptor blocker; Olmesartan medoxomil; Hypoxia; Remodeling; Collagen; Brain natriuretic peptide; TGF-β

1. Introduction

Hypoxic cor pulmonale is associated with severe chronic hypoxic lung disease with pulmonary hypertension, elevated pulmonary vascular resistance, right ventricular dysfunction, reduced oxygen transport, and decreased cardiac output (Fowler et al., 1952). Hypoxic cor pulmonale is caused by chronic hypoxia, which is the most effective and consistent stimulus for development of pulmonary hypertension, resulting in hypoxic cor pulmonale. Thus, therapy to reduce the load for right ventricular systolic function, although just symptomatic treatment, has been carried out in patients with hypoxic pulmonary disease.

Patients have been treated with various drugs to evaluate their efficacy against hypoxic pulmonary diseases (Kiely et al., 1997), and oxygen therapy has been examined (Levine et al., 1967; The Medical Research Council Working Party, 1981; Timms et al., 1985). Experimental systems to produce chronic hypoxic rats have been established; by hypobaric hypoxia (Zakheim et al., 1975; Soma et al., 1999; Resta et al., 1999), normobaric hypoxia (Pfeifer et al., 1997; Blumberg et al., 2001) and drugs (Voelkel et al., 1994; Mitani et al., 1997; Ueno et al., 2002).

In patients with hypoxic cor pulmonale, the renin–angiotensin–aldosterone system is active; angiotensinogen is converted to angiotensin I by plasma renin enzyme, angiotensin I is converted to angiotensin II by angiotensin-converting enzyme, angiotensin II induces endothelin production from endothelial cells, and endothelin induces the proliferation of smooth muscle cells (Hong et al., 2004), resulting in right ventricular systolic dysfunction. Indeed, type I angiotensin II-

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receptor blocker, Losartan, attenuates acute hypoxic pulmonary vasoconstriction in orally administered subjects (Kiely et al., 1997), and similarly an inhibitor of angiotensin I-converting enzyme prevents pulmonary vascular changes of chronic alveolar hypoxia in rats (Zakheim et al., 1975). Additionally, plasma concentrations of aldosterone were increased in patients (Farber et al., 1977), and olmesartan medoxomil, an angiotensin II-receptor blocker, was recently examined for treatment of hypertension (Brunner et al., 2003; Ishida et al., 2003; Chapell et al., 2003).

To evaluate the efficacy of olmesartan medoxomil for hypoxic pulmonary hypertension, we established a new in vivo normobaric hypoxic experimental system and used it to determine that olmesartan medoxomil has significant efficacy in hypoxic rats. Because there are no drugs that have an efficacy for just pulmonary hypertension, we also examined whether olmesartan medoxomil reduces systolic blood pressure. Olmesartan medoxomil, a clinical medicine focused on angiotensin II-receptor blockade, is commercially supplied by Sankyo Pharmaceutical Co. Ltd. (Tokyo, Japan), as OLMETEC Tablets.

2. Methods

2.1. Apparatus for normobaric hypoxic air

To obtain hypoxic pulmonary hypertensive rats, a normobaric and hypoxic air-generating system was established. The reduction of oxygen concentration was carried out by adding nitrogen which was automatically adsorbed from the air by a machine made in a collaboration between Teijin Co Ltd. (Tokyo, Japan), and Iryou Iwakuni Manufacture (Iwakuni, Yamaguchi, Japan). Normobaric hypoxic air at 10% oxygen flowed at 5–7 l/min from a small inlet hole (ϕ, 10 mm) into a commercial plastic cloth box ($70\times40\times35$ cm^3; 9.8 l) with a plastic flat cover; while consumed air flowed out of the opposite side wall from two outlet holes with the same size openings as the inlet. To remove moisture coming from urine and feces in the chamber, 1 kg CaCl was placed between the draining board and the bottom of the chamber, and replaced once a week. Under this condition, the humidity of the air was kept at about 50%. Usually, two rats were fed in a chamber to maintain a steady condition. Male Wistar rats (about 250 g) were purchased from Charles River Japan, Inc., and randomly assigned to one of the groups: control group; normoxia for 6 weeks ($n=7$; hypoxia group); treated under hypoxic conditions for 6 weeks ($n=7$; drug-treated hypoxia group); hypoxia group received oral administration of olmesartan medoxomil (5 mg/kg/day) every day for 6 weeks ($n=7$). Olmesartan medoxomil dissolved in drinking water was given orally by cannulation. Olmesartan medoxomil, $C_{29}H_{30}N_6O_6$, was kindly supplied by Sankyo Pharmaceutical Co. Ltd., and the concentration used in the present study was recommended by the manufacturer. Animal experiments were conducted according to the Guide for the Care and Use of Laboratory Animals, Dokkyo University School of Medicine.

2.2. Two-dimensional echocardiography

Rats under anesthesia with diethyl ether were laid on their back and held by hand in our specially made normobaric hypoxic glove box. Two-dimensional echocardiography (Ivy, 1999) was carried out with an echocardiographic system, Model SSA-380A, Toshiba (Tokyo, Japan), and a 7 MHz probe, at 0, 2, 4 and 6 weeks under the same conditions (normobaric and hypoxic states) in the box. In the parasternal echocardiographic window, a two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscle. To estimate the increase in right ventricular systolic pressure, we calculated the ratio of the minor axis to the major axis of the left ventricle in the end-systolic phase (Ueno et al., 2002). Measurements were performed by a single observer.

2.3. Measurement of aortic pressure

Rats were anesthetized by intraperitoneal injection of pentobarbital at 30 mg/kg; a polyethylene catheter (ATOM®, 3 Fr. ϕ: 1.0 mm) was cannulated into the right carotid artery, and connected to a pressure transducer, Polygraph MIC 8600®, Fukuda Denshi (Tokyo, Japan), for measurements. Blood pressure measurements of hypoxia rats were carried out in the normobaric hypoxic box.

2.4. Organ sampling

After 6 weeks, animals were killed by decapitation and blood collected from the carotid arteries. Hearts and lungs were removed, with some samples kept in liquid nitrogen for RNA preparation, and others in 10% buffered formalin for at least 24 h; after which organs were embedded in paraffin and 5-μm sections used for various kinds of staining.

2.5. Morphological observation

Organ sections of 5-μm were stained with hematoxylin–eosin to obtain structural and pathological information, and with Elastica–Masson stain to determine whether collagen fibers had formed. Additionally, sections were immunohistochemically stained using anti-brain natriuretic peptide (BNP), transforming growth factor (TGF-β) and endothelin antibodies. Rabbit polyclonal anti-BNP, TGF-β and endothelin antibodies were purchased from Affinity Research Products Ltd. (Mamhead, UK), Santa Cruz Biotechnology, Inc., and IBL (Immuno-Biology Laboratory; Fujioka, Gunma, Japan), respectively. Briefly, sections were incubated with the primary antibody for 1 h and then incubated with the secondary antibody, followed by ABC reagents. Color development was carried out by incubating with diamino benzidine as a substrate. Slides were counter stained with Mayer hematoxylin. Preincubation of the primary antibody with specific blocking peptides or substitution of the primary antibody with an irrelevant IgG served as negative controls.

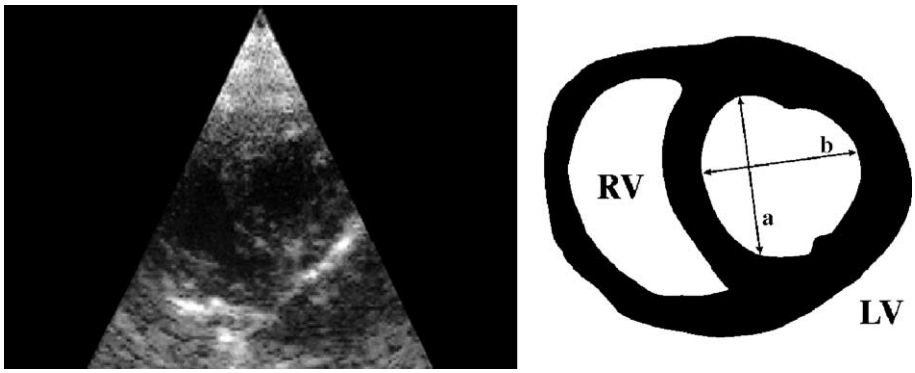

Fig. 1. Parasternal short-axis view by two-dimensional echocardiography, and an index of pulmonary hypertension in the parasternal short-axis of the left ventricle. RV = right ventricle, LV = left ventricle.

2.6. Real-time polymerase chain reaction (real-time PCR)

Total RNA was extracted from tissues by the acid guanidium–isothiocyante phenol method (Chomezynski and Sacchi, 1987), using the ISOGEN reagent, purchased from Wako Pure Chemical Co. Ltd. (Osaka, Japan). To measure BNP, TGF-β and endothelin mRNA levels, real-time PCR was assayed with a Gene Amp 5700®, Applied Biosystems (Tokyo, Japan). Complementary DNA was synthesized from a portion (1 μg) of total RNA with a TAKARA RT–PCR assay kit, TaKaRa Biomedicals (Osaka, Japan). *Taq*man probes and primers were as follows: GAPDH, (forward primer) 5′–AGCCCAGGATGCCCTTTAGT–3′, (reverse primer) 5′–CATGCCGCCTGGAGAAAC–3′, (*Taq*man probe) 5′–CCTTCACCACCTTCTTGAT–3′; BNP, (forward primer) 5′–AGAACTTCTAAAAAGAGTCCTTAGGTCTCA–3′, (reverse primer) 5′–GTGCCATCTTGGAATTTCGAA–3′, (*Taq*man probe) 5′–ACAGCGCCTTCCGGATCCAGGA–3′; TGF-β, (forward primer) 5′–CCTGGGCACCATCCATGA–3′, (reverse primer) 5′–CAGGTGTTGAGCCCTTTCCA–3′, (*Taq*man probe) 5′–CCGACCCTTCCTGCTCCTCATGG–3′; endothelin, (forward primer) 5′–AGACCCCGCAGGTCCAA–3′, (reverse primer) 5′–TGATGTCCAGGTGGCAGAAGTA–3′, (*Taq*man probe) 5′–CGTTGCTCCTGCTCCTCCTTGATGG–3′. These primers and Taqman probes were designed and obtained from Applied Biosystems (Branchburg, NJ, USA). The assays were carried out according to the protocol recommended by the manufacturer. A portion of newly synthesized cDNA was mixed with Taqman universal PCR master mix purchased from Applied Biosystems, and the mixture then applied to the apparatus.

2.7. Statistical analysis

All data were expressed as the mean ± S.D. All statistical comparisons were performed on a computer using Statview Version 5.0 for Windows XP. Significances of difference were analyzed using Kruskal–Wallis one-way analysis of variance, followed by the Fischer's test of protected least significant differences for multiple comparisons. The results were considered statistically significant at $P < 0.05$.

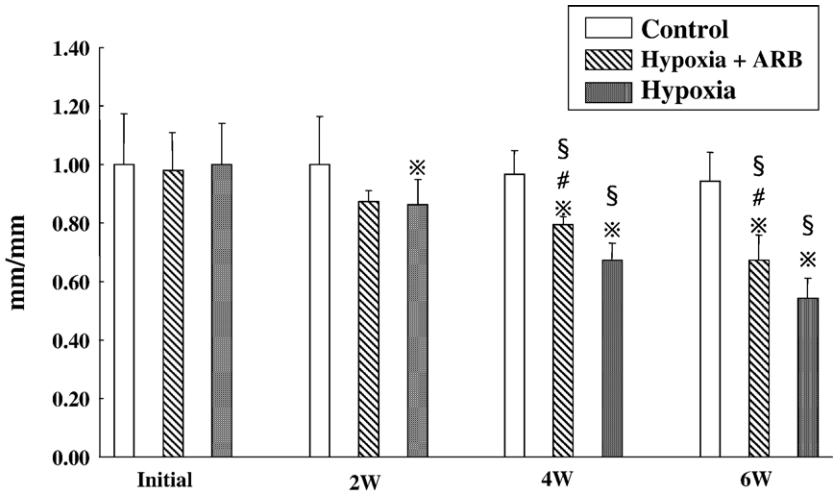

Fig. 2. Changes in the ratio of the minor axis (a) of the left ventricle (LV) to the major axis (b) of the left ventricle (LV) in rats fed under different conditions for different periods. The values of (a) and (b) were measured by two-dimensional echocardiography, and each bar represents the mean ± S.D. based on 7 rats. ARB is angiotensin receptor blockade. ※$P < 0.05$ vs. initial level, §$P < 0.05$ vs. control, #$P < 0.05$ vs. hypoxia.

3. Results

3.1. Echocardiography

Echocardiography is useful to evaluate the presence, etiology, and severity of pulmonary hypertension (Ivy, 1999). Changes in the shape of the interventricular septum provided semi-quantitative information about the severity of pulmonary hypertension. Indeed, the degree of curvature of the interventricular septum provided a marker of right ventricular systolic hypertension. Therefore, the ratio of the minor axis (*a*) of the left ventricle to the major axis (*b*) of the left ventricle was measured as an index of pulmonary hypertension (Fig. 1).

Fig. 2 shows the ratios (*a*/*b*) of the two axes at weeks 0, 2, 4 and 6 after the start of treatment. Under hypoxic conditions for 2 weeks, the overload of the right ventricular pressure induced a significant reduction of the major axis of the left ventricle, followed by a decrease in the ratio of the two axes. Further decreases in the ratio were observed after 4 and 6 weeks of treatments (Fig. 2). Thus, the hypoxic condition induced obvious hypoxic pulmonary hypertension in a time-dependent manner in rats. On the other hand, oral administration of olmesartan medoxomil significantly inhibited the effect of hypoxic treatments, although this drug could not completely abolish the hypoxic effect as seen in the ratio of the two axes.

To confirm the results obtained from echocardiography, the weights of the right and left ventricles were measured, and the ratios of these two organ part weights were calculated. In the hypoxia group, this value (0.52±0.09) was much larger than that of the controls (0.21±0.02), while in the hypoxia group treated with olmesartan medoxomil, the value was significantly reduced to 0.34±0.07 after 6 weeks of treatment. These results are consistent with those obtained from echocardiography (Fig. 2).

3.2. Blood pressure

To determine whether olmesartan medoxomil affects aortic pressure as well as pulmonary hypertension, we measured carotid aortic pressure of rats fed under different conditions. Carotid aortic pressure was found to be independent of olmesartan medoxomil among rat groups which were fed under different conditions (Table 1). Thus, olmesartan medoxomil has an efficacy for just pulmonary hypertension induced by hypoxic conditions in rats.

Table 1
Effects of olmesartan medoxomil on carotid aortic pressure in rat

	Systolic (mmHg)	Diastolic (mmHg)	Mean (mmHg)	(Number)
Normoxia	157±12	119±8	136±8	(4)
Normoxia+drug	145±20	120±18	133±20	(4)
Hypoxia	146±44	112±29	128±36	(3)
Hypoxia+drug	147±33	113±18	129±24	(4)

Drug means olmesartan medoxomil. Carotid aortic pressure was measured after 6 week feeding under various conditions. The figure in parentheses is the number of rats examined.

3.3. Pathological changes

Heart sections of 5-μm were stained with hematoxylin eosin. Hypoxic exposure of rats induced obvious hypertrophy of the right ventricle (Fig. 3-1B), while the oral administration of olmesartan medoxomil inhibited these morphological changes (Fig. 3-1C). The hypertrophy of the right ventricle induced by hypoxic exposure was reduced by the drug administration.

Sections were stained by the Elastica–Masson method to investigate whether enlargement and hypertrophy of the right ventricle were due to collagen fiber formations. A significant increase in collagen fibers was observed in the hypoxic group (Fig. 3-2B). Similarly, collagen mRNA levels were elevated in medial smooth muscle cells of new borne calves fed at a high altitude (Cruch et al., 1989). The oral administration of olmesartan medoxomil obviously inhibited collagen fiber formation (Fig. 3-2C). Chronic hypoxic exposure of rats also induced obvious collagen fiber formation in pulmonary arteries (Fig. 3-3B). In addition, hyperplasia of smooth muscle cells of bronchioles, and the prominent fibrosis of peribronchioles and pulmonary vein were observed. Similarly, this collagen fiber formation was almost completely inhibited by olmesartan medoxomil (Fig. 3-3C).

Brain natriuretic peptide (BNP) is constitutively expressed within cardiocytes in the adult mammalian heart and is released together with atrial natriuretic peptide (ANP) with which it contributes to the maintenance of homeostasis. To determine whether changes in the level of BNP were induced in the hypoxic group, heart sections were immunohistochemically stained with its antibody. Hypoxic exposure clearly increased the number of cells colored brown based on BNP production in the right RV (Fig. 4-1B), while the brown color almost disappeared in the hypoxia group treated with olmesartan medoxomil (Fig. 4-1C). Serum BNP concentration was elevated in the hypoxia group, whereas olmesartan medoxomil significantly reduced the elevated serum BNP concentration in the drug-treated group (Table 2).

TGF-βs, particularly TGF-β, regulate smooth muscle cell proliferation and vascular remodeling (Blobe et al., 2000). The production of TGF-β, which affects collagen fiber formation, was investigated immunohistochemically. Hypoxic exposure of rats increased the number of cells colored brown in the right ventricle (Fig. 4-2B), while the brown color diminished in the hypoxia group treated with the drug (Fig. 4-2C), thus demonstrating that olmesartan medoxomil inhibits the production of TGF-β induced by chronic hypoxic exposure of rats.

The effect on lungs of the hypoxic exposure of rats was also investigated pathologically. Fig. 5-1A, B and C show pulmonary arteries, pulmonary parenchyma and interstitial tissues. The production of TGF-β was also strongly induced in the endothelium of pulmonary arteries, a portion of type II epithelial cells, alveolar capillary endothelium and fibroblasts in the interstitial tissues in hypoxic rats. However, olmesartan

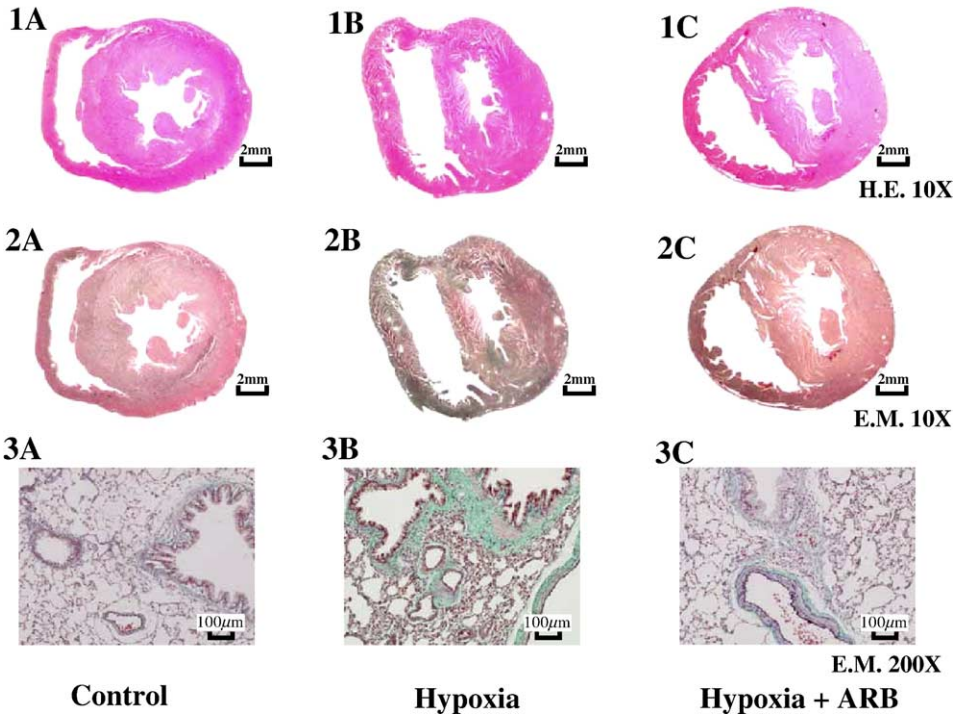

Fig. 3. Morphological changes in hearts and pulmonary arteries of rats fed under different conditions. Rats fed under different conditions for 6 weeks were killed, and fixed heart sections were stained with hematoxylin eosin (1A, 1B and 1C) or with Elastica–Masson method (2A, 2B and 2C). The fixed lung sections were stained by Elastica–Masson method (3A, 3B and 3C). 1A, 2A and 3A: control group; 1B, 2B and 3B: hypoxia group; and 1C, 2C and 3C: hypoxia group treated with olmesartan medoxomil (ARB). Microscopic magnification of heart sections was 10×, and that of lung sections 200×.

medoxomil reduced the induction of TGF-β production in the hypoxia group (Fig. 5-1C). These results indicate that the drug has an efficacy for pulmonary remodeling, as was also observed in the heart (Figs. 1–3).

Endothelin (Yanagisawa et al., 1988) has three isoforms (endothelin-1, -2 and -3) (Weissberg et al., 1990), and the endothelial family is expressed not only in vascular endothelial cells but also in other tissues. Endothelins affect via an

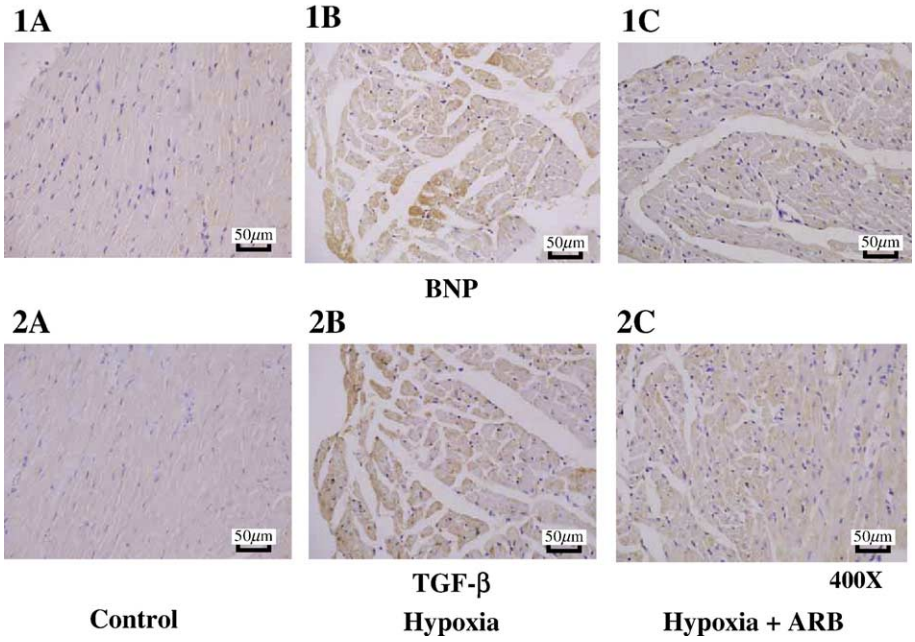

Fig. 4. Immunohistochemical staining of right ventricular cells. Sections were first treated with anti-brain natriuretic peptide (BNP) antibody (1A, 1B and 1C), or anti TGF-β antibody (2A, 2B and 2C), and then treated with the secondary antibody. 1A and 2A: control group; 1B and 2B: hypoxia group; and 1C and 2C: hypoxia group treated with olmesartan medoxomil (ARB). Rats were fed under different conditions for 6 weeks. Microscopic magnification was 200×.

Table 2
Effects of olmesartan medoxomil on serum BNP concentrations in rat

	BNP (pg/ml)	(Number)
Normoxia	47±2	(3)
Hypoxia	88±5	(3)
Hypoxia+drug	68±6[a]	(7)

Drug means olmesartan medoxomil. After rats were fed under different conditions for 6 weeks, they were killed to collect blood. The concentration of serum BNP was measured by SRL Assay Company, Tokyo, Japan, using radioimmunoassay. The figure in parentheses is the number of rats examined.
[a] *t*-Test of hypoxia vs. hypoxia+olmesartan medoxomil was at $P<0.001$.

autocrine or paracrine mechanism (Perez et al., 2003; Tikkanen et al., 2004). Endothelin induction by hypoxic exposure of rats and the effect of olmesartan medoxomil on the pulmonary remodeling were investigated. The production of endothelin in the endothelium and smooth muscle cells of pulmonary arteries as well as in the alveolar capillary endothelium was obviously induced by the hypoxic exposure of rats (Fig. 5-2B), while this induction was clearly reduced by the drug (Fig. 5-2C).

3.4. Real-time PCR

Gene expression was assayed by real-time PCR. BNP mRNA level was significantly increased in the right ventricle cells by hypoxic exposure (Fig. 6). The BNP mRNA level in the hypoxia group was 2.4-fold compared with the controls. This increase was completely inhibited by oral administration of olmesartan medoxomil into rats exposed to hypoxic air (Fig. 6A). These results are consistent with those obtained from immunohistochemical observations (Fig. 4). Thus, it was shown that olmesartan medoxomil regulates at the level of BNP transcription.

Levels of TGF-β mRNA were also investigated among the three rat groups. The hypoxic exposure significantly induced an increase in TGF-β mRNA levels in right ventricular cells, and a 2.02-fold increase was observed in the hypoxia group compared with controls (Fig. 6). This increase in TGF-β mRNA level was also abolished by administration of olmesartan medoxomil, as observed immunohistochemically (Fig. 4). These results indicate that hypoxic exposure of rats induces TGF-β at the transcriptional level in right ventricular cells and that the induction is completely inhibited by oral administration of olmesartan medoxomil.

Gene expression of endothelin in the pulmonary arteries was also investigated in the three groups. The endothelin mRNA level was 2.81-fold increased in the hypoxia group (Fig. 6), while this increase was completely abolished by oral administration of olmesartan medoxomil in the hypoxia group. These results are also consistent with those obtained from immunohistochemical observations (Fig. 5-2). Similarly, endothelin mRNA level was increased in the pulmonary arteries in the hypoxia group, and again this increase was completely inhibited by oral administration of olmesartan medoxomil (Fig. 6).

4. Discussion

Hypoxic pulmonary hypertension induces obvious hypoxic cor pulmonale in vivo (Fowler et al., 1952). Indeed, chronic hypoxic exposure of rats induced hypertrophy of the right ventricle in the present study (Figs. 1–3). Hypertrophy of the right ventricle was accompanied by the flattening of the medial

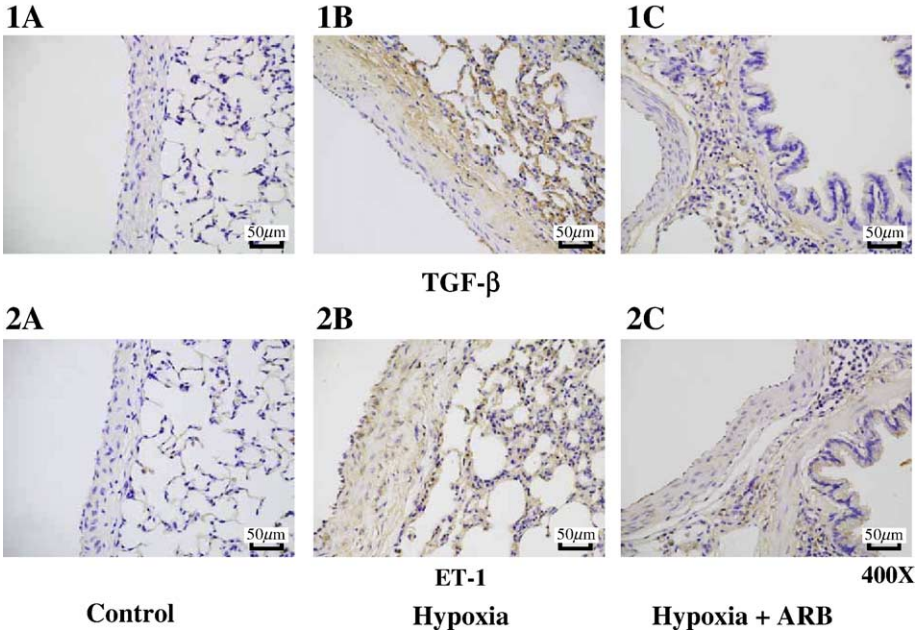

Fig. 5. Immunohistochemical staining of pulmonary arteries and pulmonary interstitial tissues. The sections were first stained with anti-TGF-β antibody (1A, 1B and 1C), or with endothelin-1 antibody (2A, 2B and 2C), and then treated with the second antibody. 1A and 2A: control group; 1B and 2B: hypoxia group; and 1C and 2C: hypoxia group treated with olmesartan medoxomil (ARB). Rats were fed under different conditions for 6 weeks. Microscopic magnification was 400×.

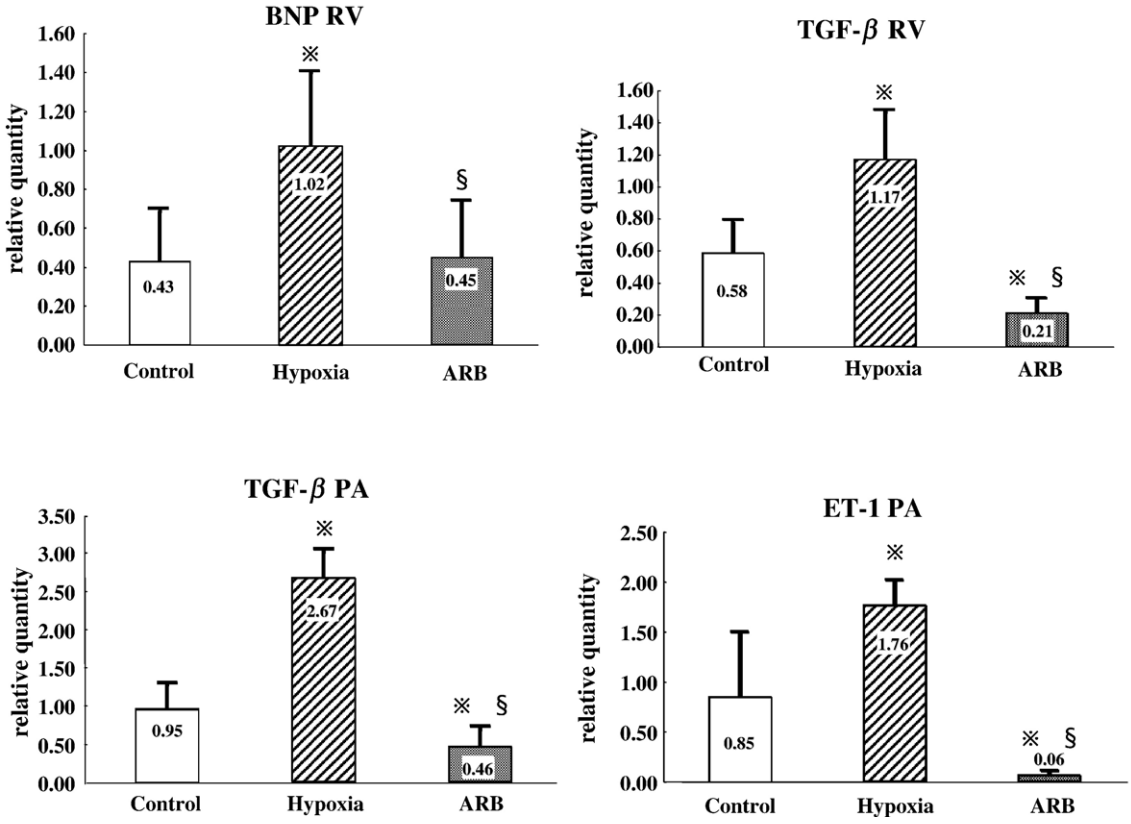

Fig. 6. Effects of olmesartan medoxomil on gene expressions in right ventricles (RV) and pulmonary arteries (PA) of rats fed under different conditions. ARB: hypoxia group was treated with olmesartan medoxomil. Rats were fed under different conditions for 6 weeks. The levels of BNP, TGF-β, endothelin-1 and GAPDH were measured by real-time PCR, and the values of BNP, TGF-β and endothelin-1 were divided by those of GAPDH. Each bar represents the mean ± S.D. based on 7 rats. ※$P<0.05$ vs. control, §$P<0.05$ vs. hypoxia.

wall in hypoxic rats, while oral administration of olmesartan medoxomil clearly inhibited these morphological changes in the right ventricle. Thus, olmesartan medoxomil efficiently prevents the pulmonary vascular changes induced by hypoxic pulmonary hypertension. Similar results were obtained from oral administration of an endothelin-receptor antagonist and/or prostacyclin analogue in drug-induced pulmonary hypertension rats (Ueno et al., 2002; O'Callaghan and Gaine, 2004).

The renin–angiotensin–aldosterone system was seen as an important pathway to induce hypoxic cor pulmonale (Farber et al., 1977). In this system, endothelin produced from endothelial cells finally induces the proliferation of smooth muscle cells followed by contraction and hypertrophy of the right ventricle. Therefore, the inhibition of the angiotensin-converting enzyme has an efficacy for hypoxic pulmonary hypertension (Zakheim et al., 1975); the angiotensin II-receptor blocker, Losartan, shows similar functions (Kiely et al., 1997). In the present study, olmesartan medoxomil, an angiotensin II-receptor blocker, clearly inhibited right ventricular hypertrophy and also inhibited increases in several gene mRNA levels in the hypoxic rats.

BNP and ANP play major roles in the salt-water homeostasis of an organism, resulting in the control of blood pressure. Oxygen tension regulates ANP secretion from the heart, and hypoxia stimulates ANP secretion both in vivo (Baertschi and Teague, 1989) and in vitro (Tóth et al., 1994). In addition, BNP is an excellent marker for the presence of pulmonary hypertension in patients (Leuchte et al., 2004). On the other hand, exogenous BNP infusion inhibits hypoxic pulmonary hypertension in rats (Klinger et al., 1998). Thus, the regulatory mechanisms of BNP may be involved in pulmonary vascular responses to hypoxia (Nagaya et al., 1988).

Based on immunohistochemical and molecular studies, olmesartan medoxomil clearly inhibited BNP synthesis in the right ventricle in hypoxia rats (Figs. 4-1 and 6). Consistent with our present data, other groups have also shown that prolonged hypoxia increased ANP mRNA levels in the right atrium and ventricle (Hill et al., 1994). Additionally, olmesartan medoxomil obviously inhibited BNP gene expression by prolonged hypoxia in rats.

It is possible to speculate that the overload of the right ventricle under hypoxia conditions passively induces hypertrophy of the right ventricle and medial wall. However, these changes were not only due to hypertrophy but also to the strengthening of tissues as collagen fiber formations were observed (Fig. 3). Collagen fiber formation was also induced in pulmonary arteries (Fig. 3). In addition, based on our immunohistochemical and molecular studies, the production of TGF-β, which activates collagen gene expression (Sarkar et al., 2004), was increased by hypoxic exposure of rats (Figs. 4–6). These results indicate that organisms actively strengthen both right ventricular and pulmonary artery structures to resist high

pulmonary arterial pressure in pulmonary hypertension; which is followed by expressions of several genes.

Endothelin is one of the factors that activate smooth muscle cells; thus, endothelin secretion induces proliferation of smooth muscle cells, resulting in hypertrophy of the right ventricle. Indeed, endothelin production was observed in the endothelial layer of the pulmonary arterial wall in hypoxia rats based on immunohistochemical analysis (Fig. 5-2), and molecular studies (Fig. 6). Thus, endothelin secretion from vascular endothelial cells seems to be due to active resistance to hypoxic pulmonary arterial hypertension. As olmesartan medoxomil inhibited increases in the production of both BNP and TGF-β or in their mRNA levels, this drug clearly inhibited either endothelin production or its mRNA level in hypoxic rat. Olmesartan medoxomil reduced TGF-β and endothelin to levels below those seen in mRNA levels in control rats. However, olmesartan medoxomil only partially abolish the hypoxic effects in echocardiographic changes and in immunohistochemical stainings for TGF-β and endothelin. Thus, the amounts of these compounds were not quantitatively correlated to those of mRNAs. Similar olmesartan medoxomil effects were observed in collagen and BNP. It is possible that these gaps are based on translation efficiencies. The present results indicate that olmesartan medoxomil has good efficacy towards hypoxic pulmonary hypertension. Similarly, it has an efficacy towards hypertension (Brunner and Laeis, 2003; Ishida et al., 2003; Chapell et al., 2003) and, in our study, left ventricular changes based on hypoxic cor pulmonale were also recovered through treatment with this drug (unpublished data). However, the present study shows that olmesartan medoxomil does not affect the normal cardiovascular system. Additionally, we observed no apparent side effects of olmesartan medoxomil administration in the present study; thus, olmesartan medoxomil seems to be efficient against various vascular failures induced by hypertension.

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