

# Mesenteric artery remodeling and effects of imidapril and irbesartan on it in spontaneously hypertensive rats

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## Abstract

**AIM:** To investigate the remodeling of mesenteric artery and the expression of TGF- $\beta_1$ , c-Jun in mesenteric artery and effects of imidapril and irbesartan on the remodeling in spontaneously hypertensive rats (SHR).

**METHODS:** Thirty SHR (male/female, 21/9), aged 13 wk, were randomly divided into 3 groups (7 male rats and 3 female rats each group): SHR group, imidapril group (imidapril 3 mg/kg·d was given in drinking water for 14 wk), and irbesartan group (irbesartan 50 mg/kg·d was given in drinking water for 14 wk). Ten homogenous Wistar Kyoto rats, 5 males and 5 females, weighing 206±49 g, were selected as normal control group (WKY group). Systolic pressure was measured on d 1, 2, 4, 6, 8, 10, 12 and 14 during the experiment and the rats were killed at the end of the experiment. Angiotensin II (Ang II) level in plasma and mesenteric arteries was measured by radioimmunoassay. The morphology of the secondary branches of mesenteric artery were examined by light microscopy and electron microscopy. Reverse transcription polymerase chain reaction (RT-PCR) was used to detect the expression of transforming growth factor TGF- $\beta_1$  and c-Jun mRNA.

**RESULTS:** Compared with imidapril group and irbesartan group, the blood pressure was remarkably increased in SHR group. Ang II level in plasma and mesenteric arteries in SHR group was the same or lower than that in WKY group, and was higher in irbesartan group and lower in imidapril group. The remodeling of mesenteric arteries in SHR group was mostly obvious among the 4 groups. The ratio of TGF- $\beta_1$  absorbed light value to GAPDH absorbed light value in the SHR group was 0.887±0.019, which was significantly higher than that in WKY group, imidapril group, and irbesartan group with the ratios of 0.780±0.018, 0.803±0.005, and 0.847±0.017, respectively ( $P<0.01$ ). Ang II level in plasma and mesenteric arteries in imidapril group was significantly lower than that in irbesartan group ( $P<0.05$ ). The c-Jun absorbed light value/GAPDH absorbed light value of mesenteric arteries in the SHR group was 0.850±0.015, which was significantly higher than that in the WKY, imidapril, and irbesartan groups (0.582±0.013,

0.743±0.012, and 0.789±0.013, respectively,  $P<0.01$ ), and was significantly lower in imidapril group than in irbesartan group ( $P<0.05$ ).

**CONCLUSION:** Imidapril and irbesartan can not only control blood pressure but also inhibit mesenteric arteries remodeling and mRNA expression of TGF- $\beta_1$ , c-Jun in SHR. Imidapril is more effective than irbesartan.

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## INTRODUCTION

It has been reported<sup>[1-3]</sup> that angiotensin-converting enzyme inhibitor (ACEI) and angiotensin II type 1 (AT1) receptor antagonist can inhibit resistance blood vessel remodeling, but their action mechanism is still unknown. We selected irbesartan and imidapril to interfere mesenteric artery remodeling in spontaneously hypertensive rats (SHR) to investigate the expression of c-Jun and TGF- $\beta_1$  mRNA in resistance blood vessel of each group rats with reverse transcription polymerase chain reaction (RT-PCR) and to illustrate the mechanism of resistance blood vessel remodeling in hypertension and possible mechanism of these two drugs inhibiting mesenteric artery remodeling and possible effect on the inhibition of mesenteric artery remodeling.

## MATERIALS AND METHODS

### Materials

Thirty 13-wk old SHR (male/female, 21/9, provided by Fuwai Hospital in Beijing) with an average body mass of 228±39 g were randomly divided into 3 groups: SHR positive control group, imidapril treatment group (3 mg/kg·d), irbesartan treatment group (50 mg/kg·d). Ten homogenous Wistar-Kyoto rats [provided by Fuwai Hospital in Beijing, in which female rats were 5, male rats were 5, their average body mass was 206±49 g.] were selected as normal control group. During the 14-wk trial, all rats were in the breeding conditions: temperature 18-25 °C, humidity 40-60%, protein feed concentration 22-25%.

### Methods

Irbesartan (presented by Hengrui Pharmacy Factory of Jiangsu Province) 50 mg/kg·d<sup>[4]</sup> and imidapril (presented by Tianbian Pharmacy Factory of Tianjin) 3 mg/kg·d<sup>[5]</sup> were dissolved in drinking water for 14 successive wk, respectively. Index observed included tail artery systolic blood pressure, angiotensin II (Ang II), histology of mesenteric artery. Fourteen weeks after irbesartan and imidapril interfering, all rats were killed and the second grade embranchment of mesenteric artery (about 2 mm) was taken and put into 25/L of glutaral for fixing, then transmission electron microscope (H-600, Hitachi in Japan) was used. About 1 mm of the artery was put into 100 g/L of neutral

formaldehyde and stained with HE, then observed by light microscope. Morphology of mesenteric artery was by a computer-assisted image analysis system. RT-PCR analysis was performed for TGF- $\beta_1$ <sup>[6]</sup> and c-Jun<sup>[7]</sup> mRNA level in mesenteric artery.

SPSS 10.0 statistically analyzed the data and results were expressed as mean $\pm$ SD.

## RESULTS

*Blood pressure from 4 groups was recorded in Table 1*

*Concentration of Ang II in plasma is shown in Table 2*

### *Morphology of mesenteric artery (Figure 1)*

Intima, vessel media, vessel wall were not increased in WKY group. Vessel lumen was relatively wider (A). Intima, vessel media and vessel wall were increased. Vessel lumen was relatively narrow in SHR (B), imidapril (C) and irbesartan (D) groups. The ratios of intima-media thickness / lumen radius, media / lumen area, lumen / vessel radius in 4 groups are shown in Table 3.

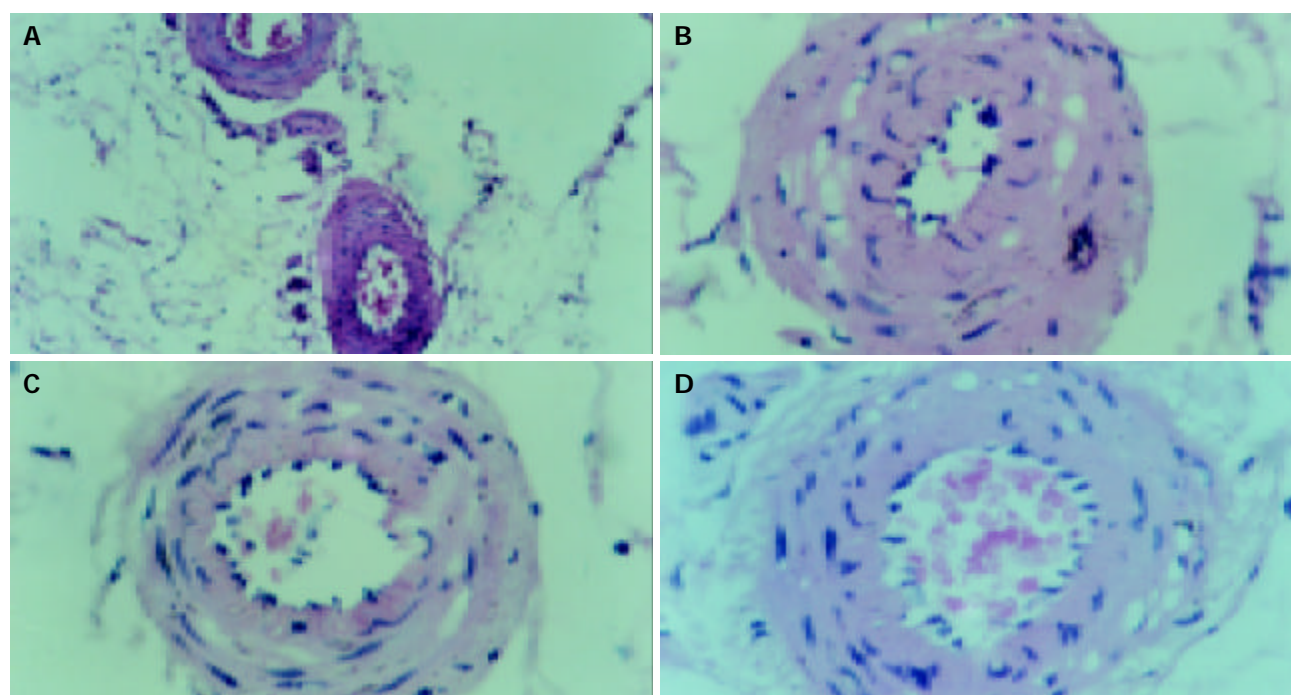
### *Microstructure of mesenteric arteries (transmission electron microscope, Figure 2)*

As shown in A for WKY group, endothelial cells of intima were

abundant and normal, media had more smooth muscle cells. As shown in B for SHR group, endothelial cells of intima had vacuoles and fibrous tissues with adventitial hyperplasia, the thickness of adventitia was increased, media was severely fibrous and the fibrous tissue extended to smooth muscle layer and invaded internal elastic lamina, internal elastic lamina was tortuous and atrophic, some of smooth muscle cells were replaced by fibrous tissue. As shown in C for irbesartan group, endothelial cells of intima had vacuoles and marrow type corpses, internal elastic lamina was tortuous and atrophic and infiltrated by collagen fibers, but fibrosis in whole blood vessel wall relieved as compared with SHR group. The number of smooth muscle cells in media was slightly more than that in SHR group. As shown in D for imidapril group, endothelial cells of intima had marrow type corpses but no vacuole, the number of smooth muscle cells in media was more than that in irbesartan group, internal elastic lamina had a close-to-normal distribution, local internal elastic lamina was narrowed, fibrosis did not occur on blood vessel walls.

### *RT-PCR analysis of TGF- $\beta_1$ and c-Jun mRNA level in mesenteric artery (Figure 3)*

mRNA expression levels of TGF- $\beta_1$  and c-Jun were analyzed by RT-PCR. Agarose gel electrophoresis of the PCR products was carried out to measure the relative intensity of the expression (A and B, Table 4).

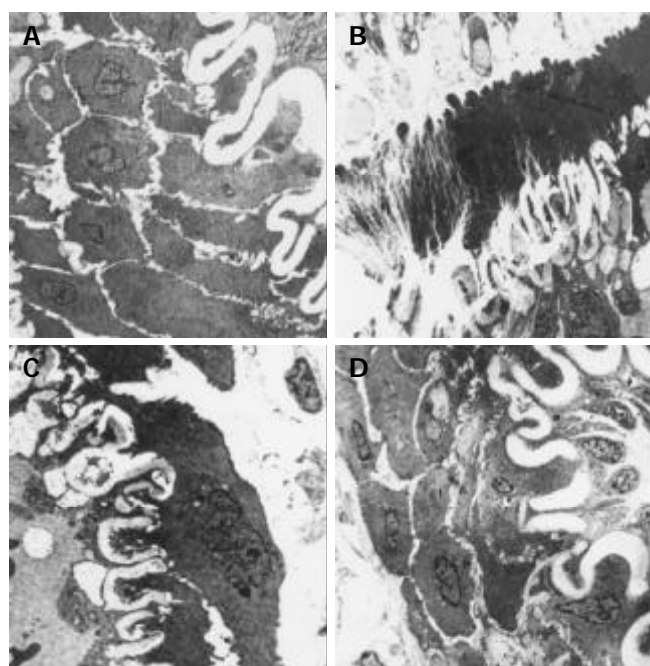


**Figure 1** Morphology of mesenteric artery, A: Intima, media of vessel wall are not increased in WKY group and vessel lumen is relatively wider (HE,  $\times 100$ ). B: Intima, media of vessel wall are increased in SHR group and vessel lumen is relatively narrow (HE,  $\times 400$ ). C: Intima, media of vessel wall are increased in imidapril group and lumen of vessel is relatively narrow (HE,  $\times 400$ ). D: Intima, media of vessel wall are increased in irbesartan group and lumen of vessel is relatively narrow (HE,  $\times 400$ ).

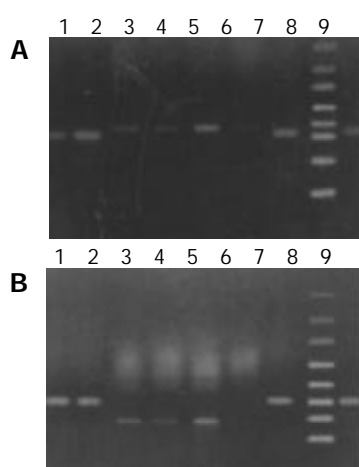
**Table 1** Blood pressure measured in WKY, SHR, imidapril and irbesartan groups (mean $\pm$ SD,  $n=10$ )

Group	Blood pressure (mmHg)							
	13-wk-old	14-wk-old	15-wk-old	17-wk-old	19-wk-old	21-wk-old	24-wk-old	26-wk-old
WKY	105.90 $\pm$ 16.10 <sup>d</sup>	115.70 $\pm$ 9.19	112.00 $\pm$ 7.53	90.00 $\pm$ 9.13	125.50 $\pm$ 7.62	116.00 $\pm$ 6.99	116.00 $\pm$ 11.25	121.50 $\pm$ 4.74
SHR	134.40 $\pm$ 7.72	140.00 $\pm$ 17.48	151.00 $\pm$ 24.59 <sup>b</sup>	160.00 $\pm$ 14.90 <sup>f</sup>	177.00 $\pm$ 16.19 <sup>f</sup>	177.50 $\pm$ 14.39 <sup>f</sup>	190.00 $\pm$ 19.00 <sup>f</sup>	198.10 $\pm$ 14.04 <sup>f</sup>
Imidapril	131.50 $\pm$ 6.68	124.30 $\pm$ 7.02	127.50 $\pm$ 8.58	125.00 $\pm$ 7.45	130.80 $\pm$ 15.16	138.00 $\pm$ 12.52	127.00 $\pm$ 11.10	142.00 $\pm$ 6.32
Irbesartan	140.10 $\pm$ 5.90	132.50 $\pm$ 15.14	124.00 $\pm$ 18.83	122.50 $\pm$ 20.17	128.00 $\pm$ 12.06	138.00 $\pm$ 6.32	126.00 $\pm$ 6.15	141.00 $\pm$ 14.87

<sup>b</sup> $P<0.01$  vs WKY, imidapril, irbesartan groups; <sup>d</sup> $P<0.01$  vs SHR, imidapril, irbesartan groups; <sup>f</sup> $P<0.01$  vs WKY, imidapril, irbesartan groups.



**Figure 2** Microstructure of mesenteric arteries (transmission electron microscope). A: WKY group, endothelial cells of intima were abundant and normal, Media has more smooth muscle cells, internal elastic lamina is normal ( $\times 2\,000$ ). B: SHR group, endothelial cells of intima had vacuole and fibrous tissue with adventitial hyperplasia; thickness of the adventitia was increased; media was severely fibrous and the fibrous tissue extended to smooth muscle layer and invaded internal elastic lamina; internal elastic lamina was tortuous and atrophic; some of smooth muscle cells were replaced by fibrous tissue ( $\times 2\,500$ ). C: Irbesartan group, endothelial cells of intima have vacuole and marrow type corpses, internal elastic lamina was tortuous and atrophic and infiltrated by collagen fibers ( $\times 2\,500$ ). D: Imidapril group, endothelial cells of intima have marrow type corpses but no vacuole, numbers of smooth muscle cells in media were more than those in irbesartan group; internal elastic lamina got a close-to-normal distribution; local internal elastic lamina got narrow, fibrosis did not occur on blood vessel wall ( $\times 2\,000$ ).



**Figure 3** Agarose gel electrophoresis of TGF- $\beta_1$  and c-Jun mRNA RT-PCR product of mesenteric arteries. A: Agarose gel (2%) electrophoresis of TGF- $\beta_1$  mRNA RT-PCR product of mesenteric arteries of 4 groups. 1: WKY group GAPDH, 2: SHR group GAPDH, 3: irbesartan group, 4: imidapril group, 5: SHR group, 6: WKY group, 7: irbesartan group GAPDH, 8: pGEM-7Zf(+)/Hae III markers (from top to bottom: 102, 142, 174, 267, 289, 328, 434, 657 bp), 9: imidapril group GAPDH. B: Agarose gel (2%) electrophoresis of c-Jun mRNA RT-PCR product of

mesenteric arteries of 4 groups. 1: SHR group GAPDH, 2: WKY group GAPDH, 3: irbesartan group, 4: imidapril group, 5: SHR group, 6: WKY group, 7: irbesartan group GAPDH, 8: pGEM-3Zf(+)/Hae III markers (from top to bottom: 18, 80, 102, 174, 267, 314, 434, 587 bp), 9: imidapril group GAPDH.

**Table 2** Concentration of Ang II in plasma (mean $\pm$ SD,  $n=7$ )

Group	Plasma (pg/mL)	Mesenteric artery (pg/100 mg)
WKY	303.15 $\pm$ 16.99	2 218.63 $\pm$ 242.37
SHR	318.77 $\pm$ 16.83	2 138.48 $\pm$ 110.56 <sup>a</sup>
Imidapril	307.43 $\pm$ 25.20	1 888.92 $\pm$ 147.46 <sup>ce</sup>
Irbesartan	571.38 $\pm$ 57.89 <sup>b</sup>	3 509.18 $\pm$ 168.44 <sup>bfg</sup>

<sup>a</sup> $P<0.05$  vs WKY, <sup>b</sup> $P<0.01$  vs WKY, SHR, imidapril group, <sup>c</sup> $P<0.05$  vs WKY, <sup>e</sup> $P<0.05$  vs SHR, <sup>f</sup> $P<0.01$  vs SHR, <sup>g</sup> $P<0.01$  vs WKY, <sup>g</sup> $P<0.05$  vs imidapril.

**Table 3** Comparisons of ratios of intima-media thickness / lumen radius, media / lumen area, lumen / vessel radius in four groups (mean $\pm$ SD,  $n=7$ )

Group	Media thickness (mm)/lumen radius (mm)	Media area (mm <sup>2</sup> )/lumen area (mm <sup>2</sup> )	Lumen radius (mm)/vessel radius (mm)
WKY	0.75 $\pm$ 0.09	0.35 $\pm$ 0.04	0.65 $\pm$ 0.01
SHR	2.67 $\pm$ 0.20 <sup>a</sup>	1.21 $\pm$ 0.14 <sup>a</sup>	0.46 $\pm$ 0.01 <sup>a</sup>
Imidapril	1.67 $\pm$ 0.13 <sup>ec</sup>	0.71 $\pm$ 0.05 <sup>ec</sup>	0.51 $\pm$ 0.01 <sup>ec</sup>
Irbesartan	1.47 $\pm$ 0.27 <sup>ge</sup>	0.65 $\pm$ 0.14 <sup>ge</sup>	0.53 $\pm$ 0.01 <sup>ge</sup>

<sup>a</sup> $P<0.05$  vs WKY; <sup>c</sup> $P<0.05$  vs SHR; <sup>e</sup> $P<0.05$  vs WKY; <sup>e</sup> $P<0.05$  vs SHR; <sup>g</sup> $P<0.05$  vs WKY.

**Table 4** Absorbance of c-Jun/absorbance of GAPDH, absorbance of TGF- $\beta_1$ /absorbance of GAPDH (mean $\pm$ SD,  $n=5$ )

Group	Absorbance of c-Jun /absorbance of GAPDH	Absorbance of TGF- $\beta_1$ /absorbance of GAPDH
WKY group	0.582 $\pm$ 0.013	0.780 $\pm$ 0.018
SHR group	0.850 $\pm$ 0.015 <sup>b</sup>	0.887 $\pm$ 0.019 <sup>d</sup>
Imidapril group	0.743 $\pm$ 0.012 <sup>a</sup>	0.803 $\pm$ 0.005 <sup>c</sup>
Irbesartan group	0.789 $\pm$ 0.013	0.847 $\pm$ 0.017

$F=1\,340$  in the ratio of absorbance of c-Jun/absorbance of GAPDH. <sup>a</sup> $P<0.05$  vs irbesartan group, <sup>b</sup> $P<0.01$  vs WKY, imidapril, irbesartan group.  $F=198$  in the ratio of Absorbance of TGF- $\beta_1$ /absorbance of GAPDH. <sup>c</sup> $P<0.05$  vs irbesartan group, <sup>d</sup> $P<0.01$  vs WKY, imidapril, irbesartan group.

## DISCUSSION

It is known that elevated blood pressure in essential hypertension patients and spontaneously hypertensive rats (SHR) related to the renin-angiotensin system (RAS)<sup>[8-10]</sup>. The main effector peptide of RAS was Ang II<sup>[11]</sup>, which played an essential role in the pathogenesis of hypertension through the regulation of cell growth, inflammation, and fibrosis<sup>[12]</sup>. The main biological effects of Ang II has been found to be the enhancement of smooth muscle contraction, aldosterone release<sup>[13]</sup>, arginine vasopressin release, cell proliferation, adjustment of body fluid balance. It had a close relation to blood vessel remodeling<sup>[14]</sup>. There were 2 main angiotensin II receptors, AT1 and AT2<sup>[15,16]</sup>. The AT1 receptor was responsible for most of the pathophysiologic actions of angiotensin II<sup>[17]</sup>, including cell proliferation, production of growth factors and cytokines, and fibrosis. AT2 could cause antiproliferation and counteract the cell growth induced by AT1 activation<sup>[18]</sup>. In addition, pressure-lowering agent also had actions on blood vessel remodeling<sup>[19]</sup>. Both

ACEI and AT1 acceptor antagonists have been shown to act selectively on different cycles and they not only had satisfactory decompression effect, but also might inhibit blood vessel remodeling<sup>[20,21]</sup>.

These results suggest that imidapril and irbesartan have ideal decompression effects and inhibiting action upon angiotensin-converting enzymes and AT1 acceptors. Blood pressure gradually rose and arrived to 200 mmHg in 26 wk-old SHR, but blood pressure in imidapril and irbesartan groups fluctuated within normal ranges and no obvious difference was observed between the 2 groups. Ang II level in plasma gradually increased in SHR group and slowly decreased in imidapril group compared with that in WKY group performed with radioimmunoassay, but there was no statistical significance between them. Ang II level in plasma increased in irbesartan group and it had a significant difference when compared with that in WKY group. Ang II level decreased in mesenteric artery in SHR group and it was obvious in imidapril group. Ang II level increased in mesenteric artery in irbesartan group and it had a significant difference. Campbell *et al.*<sup>[22]</sup> made clear with experiment that Ang II levels in SHR plasma, lung, kidney, heart, adrenal, aorta, brown adipose tissue were lower than the levels in Donryu rats. In this experiment, Ang II levels in plasma of SHR group and WKY group and mesenteric artery were consistent with Duncan's experiment. Moreover, imidapril decreased Ang II levels and irbesartan increased Ang II levels<sup>[23]</sup> in SHR plasma and mesenteric artery in this experiment.

Light microscopy and electron microscopy displayed that imidapril and irbesartan might inhibit structure alterations especially interstitial fibrosis. Furthermore, the effect of imidapril was better than that of irbesartan. It should be pointed out that electron microscopy of mesenteric artery displayed that the pathology of mesenteric artery remodeling in SHR possibly involved in fibrous tissue hyperplasia in adventitia, fibrosis in media, structure and function damage in endothelial cells. Castro *et al.*<sup>[24]</sup> believed that extracellular matrix (ECM) accumulation in blood vessel walls could be attributable to constriction of artery lumen in hypertension. The pathological changes of resistance vessels could relate to the synthesis and excretion reduction of proteoglycan in blood vessel smooth muscle cells.

Several experiments have shown that Ang II could induce vascular smooth muscle cell proliferation *in vivo*. Griffin suggested that Ang II infusion in rats increased mesenteric vascular media width, media cross-sectional area and media/lumen ratio, and these changes were not inhibited by hydralazine despite normalization of blood pressure. Kim *et al.*<sup>[25]</sup> also revealed that aortic ERK and JNK activities were significantly increased with the development of hypertension, and in particular, these activities were gradually and chronically enhanced in the development of hypertension and associated with an increase in aortic weight. *In vitro* experiments have also shown that Ang II stimulated protein synthesis and induced cellular hypertrophy in cultured vascular smooth muscle cells via AT1 receptors. More and more evidences have shown that AT1 receptors couple to a heterotrimeric G protein Gq. The activation of AT1 receptors could not only lead to the activation of PLC- $\beta$  and increases of diacylglycerol and Ca<sup>2+</sup> in cells but also activate intracellular signal transduction in cultured vascular smooth muscle cells. Schmitz *et al.*<sup>[26]</sup> reported that Ang II could also activate JNK of vascular smooth muscle cells. JNK is well known to increase c-Jun transactivation by phosphorylating c-Jun on 2 critical N-terminal serine residues and inducing *c-fos* gene expression. Therefore, it has been well known that JNK is involved in the activation of transcription factor, AP-1<sup>[27]</sup>. AP-1 is bound to TPA response component (TRE) of nucleus DNA to accelerate transcription and to increase proliferation and protein synthesis of vascular smooth

muscle cells. In vascular smooth muscle cells, activation of ERK and AP-1 could increase expression of TGF- $\beta_1$  mRNA<sup>[28]</sup>. Both vascular endothelial cells and vascular smooth muscle cells could synthesis TGF- $\beta_1$ <sup>[29]</sup>. TGF- $\beta_1$  has been found to be one kind of multi-function proteins<sup>[30]</sup> and to adjust hypertrophy and polyploidy of many kinds of cells and to stimulate and inhibit hyperplasia. In a word, it relates to vessel remodeling. TGF- $\beta_1$  is bound to specific receptors in cell surface to initiate the intracellular p53-dependent signaling cascade, resulting in down regulation or inhibition of cyclin-dependent kinases 2 and 4, and inducing cell cycle arrest in G<sub>1</sub>. Ang II can mediate intracellular signal transduction of vascular smooth muscle cells. Remodeling of mesenteric artery in SHR would be inhibited if the expression of c-Jun and/or TGF- $\beta_1$  could be inhibited. Therefore, this experiment used AT1 receptor antagonist irbesartan and ACE inhibitor imidapril to interfere mesenteric artery remodeling in SHR in order to investigate the expression difference of c-Jun and TGF- $\beta_1$  mRNA in mesenteric artery with RT-PCR and to illustrate the intervention action and effect difference of these two kinds of drugs on inhibiting mesenteric artery remodeling.

Compared with WKY group, the mRNA level of TGF- $\beta_1$  and c-Jun in mesenteric arteries of SHR group was obviously increased. Imidapril and irbesartan might inhibit the expression of c-Jun and TGF- $\beta_1$  mRNA in mesenteric artery of SHR. Imidapril was better than irbesartan in preventing mesenteric arteries from structure modulation especially fibrosis and expression of TGF- $\beta_1$ , -Jun mRNA. Ohta *et al* proved that aortic TGF- $\beta_1$ , fibronectin, and collagen type IV WmRNA levels were higher in SHR than in WKY, and all of these elevated mRNAs in the aorta of SHR were significantly reduced by an ACE inhibitor, alacepril (50 mg/kg·d), or an AT1 receptor antagonist, SC-52458 (50 mg/kg·d). Kim *et al.*<sup>[31]</sup> also showed that treatment with AT1 receptor antagonist (E4177, 20 mg/kg·d) significantly inhibited the activation of JNK and ERK in injured arteries. These experiments illuminate that the results in our investigation are acceptable. Further study should be done for the combined action of imidapril and irbesartan.

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