

The Influence of Telmisartan on the Expression of BMP-7 in Rat Kidneys Induced by 8% NaCl

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Abstract: Excessive salt intake is one of the factors of high blood pressure leading to kidney disease while telmisartan is one of the antihypertensive drugs used in therapy. Telmisartan not only blocks the angiotensin receptor, which leads to a reduction in blood pressure, but also activates the peroxisome proliferator-activated receptor gamma (PPAR- γ), inhibits the expression of transforming growth factor beta-1 (TGF β -1) and increases bone morphogenetic protein-7 (BMP-7). This experiment examines whether telmisartan increases the expression of BMP-7 in excess NaCl-induced Wistar rats. For this study, 25 male Wistars aged 2.5 to 3 months and rats weighing 100 to 150 g were used. They were grouped into 5, each consists of 5 rats. Group I (G I) as the first negative control received neither NaCl nor telmisartan. G II as the second negative control received NaCl but no telmisartan. G III, IV and V received NaCl and telmisartan 3, 6 and 12 mg/kg body weight. Treatments were given every day for 8 weeks. On day 56, all rats were sacrificed by neck dislocation and operated on to remove the kidney. BMP-7 expression was measured by immunohistochemical technique. Data were expressed as mean $\pm$ standard error. They were analyzed by parametric (ANOVA) or nonparametric (Kruskal-Wallis) testing. A value of $p \leq 0.05$ was considered statistically significant. The results showed that the expression of the intraglomerular and extraglomerular protein BMP-7 was higher in the telmisartan-treated group of Wistar rats than in the negative control group ($p < 0.05$). In summary, intraglomerular and extraglomerular BMP-7 protein expression was higher in male Wistar rats treated with telmisartan and induced with 8% sodium chloride than in negative control group members.

Keywords: NaCl; Telmisartan; BMP-7

1. Introduction

In 2010, noncommunicable diseases (NCDs) caused 36 million deaths each year, 63% of all deaths worldwide. The top three noncommunicable diseases are cancers, cardiovascular diseases and diabetes (WHO, 2010).

Essential arterial hypertension is society's greatest health problem. In 2005, approximately billion people (14%) worldwide suffered from high blood pressure. Hypertension is the main risk factor for cardiovascular, cerebrovascular and renal diseases, which are related to the occurrence of fibrosis in various organs such as heart, kidney, liver and cardiovascular (Blaustein et al., 2012 & Cox et al., 2012).

Previous studies in animal models showed that 8% sodium chloride can induce hypertension in rats (Yu et al., 1998). The mechanism is via the activation of angiotensin II by sodium in aldosterone $\rightarrow$ endogenous ouabain (EO) (Leenen, 2010). Angiotensin II stimulates vasoconstriction and the adrenal gland to secrete aldosterone, resulting in distal tubule stimulation and reabsorption of water (Jöhren et al., 2004; Starr & McMillan 2012). In addition, angiotensin II induces the switch from fibroblast to myofibroblast via transforming growth factor-beta1 (TGF β 1). The fibroblast produces an exaggerated extracellular matrix (ECM), therefore the ECM accumulates in the tubulointerstitial area (Mezzano et al., 2001). TGF- β 1 is a cytokine that has a role in fibrosis formation through decreased expression of BMP in the proximal tubular epithelium during renal fibrosis (Gould et al., 2002). Bramlage et al. (2010) found that

inhibition of fibrosis signaling pathway by TGF- β 1 can increase BMP-7 gene expression in hypertensive nephrosclerosis, tubulointerstitial fibrosis and diabetic nephropathy. Therefore, BMP-7 plays a role as an antibiotic for the kidney (Weiskirchen et al., 2009). According to Zeisberg et al. (2006) that BMP-7 is mainly found in kidney, cartilage and bone and can potentially be studied as a biomarker for efficacy and potential new effects.

Telmisartan not only blocks the angiotensin receptor, but also plays a role as a partial agonist of peroxisome proliferator-activated receptor- γ (PPAR- γ), thereby activating PPAR- γ (Chambers, 2008 & Funao et al., 2009). The activation causes PPAR- γ form a heterodimer with retinoid X receptors (RXR), creating a corepressor that can inhibit TGF- β 1 gene expression (Rotman & Wahli, 2010).

2. Materials and Methods

Twenty-five 2.5-3 month old male Wistars and 100-150 g BW rats were used in this experiment. They were housed in individual pens and provided with appropriate pelleted feed and drinking water, placed in room temperature 20-24°C, dark-light cycle for 12 hours. Before treatment with, the animal model was acclimated for 7 days. They were grouped into 5 groups each consisting of 5 rats. Group I (GI) as the first negative control received neither NaCl nor telmisartan. G II as the second negative control received NaCl but no telmisartan. G III, IV and V received NaCl and telmisartan 3, 6 and 12 mg/kg body weight. Treatments

were given each day for 8 weeks. On day 56, all rats were euthanized by dislocating the neck and performing kidney surgery (Younis et al., 2010 & Jawi et al., 2012).

Telmisartan 40 mg tablets were crushed and then add 40 ml water. Its suspension was taken with a syringe suitable for the dose of rats that was determined to be inserted directly into the stomach of rats (Xu & Liu, 2013). BMP-7 protein expression was measured using the immunohistochemistry technique and determined by measuring the area of stained tissue within a given field. The stained area was calculated using the image J software as a percentage of the total area within a field (Yu et al., 1998; 19. Lync et al, 1969; Biolegend, 2008; Fatchiyah et al., 2011).

Data are expressed as the mean $\pm$ standard error and are analyzed using a parametric (ANOVA) or non-parametric (Kruskal-Wallis) test. A value of $p \leq 0.05$ was considered statistically significant.

3. Results

Telmisartan effect to BMP-7 Protein Expression in Kidney of 8% Sodium Chloride- Induced Wistar rats
The intraglomerular and extraglomerular expression of BMP-7 protein was higher in the kidney of telmisartan-induced Wistar rats than in the negative control. According to Tables 1 and 2 and 1, the BMP protein expression is -7 intraglomerular and extraglomerular of Group III , IV and V>Group I and II.

Table 1. IntraglomerularBMP-7 protein expression (group I and II=negative control; group III, IV and V=8% NaCl+telmisartan 3, 6 and 12 mg/kg BW).

Group	BMP-7 protein expression (%) of rat					Mean $\pm$ SD	p
	Number						
	1	2	3	4	5		
I	19.8	23.8	24.2	0.38	20.4	17.71 $\pm$ 9.8	0.018*
II	22.5	29.3	32.9	19.5	14.4	23.72 $\pm$ 7.4	
III	26.8	32.1	27.4	22.1	18.1	25.3 $\pm$ 5.3	
IV	28.1	18.4	34.9	15.6	37.7	26.94 $\pm$ 9.7	
V	38.2	45.9	46.1	56.1	33.9	44.04 $\pm$ 8.5	

*significant difference of mean in Wistar rat group (p<0.05)

*significant difference of mean in Wistar rat group ($p < 0.05$)

Table 2. ExtraglomerularBMP-7 protein expression (group I and II=negative control; group III, IV and V=8% NaCl+telmisartan 3, 6 and 12 mg/kg BW)

Group	BMP-7 protein expression (%) of rat					Mean ± SD	p
	Number						
	1	2	3	4	5		
I	36.1	53.6	54.0	6.25	57.9	41.57 ± 21.4	0.025*
II	54.2	48.9	42.9	60.4	21.3	45.54 ± 15.01	
III	49.6	41	53.9	55.2	39	47.74 ± 7.3	
IV	56.7	49.9	35.6	48.4	46.2	47.36 ± 7.65	
V	59.4	63.4	63.2	63.5	68.4	63.58 ± 3.19	

*Significant difference of mean inWistar rat group (p<0.05)

*Significant difference of mean inWistar rat group ($p < 0.05$)

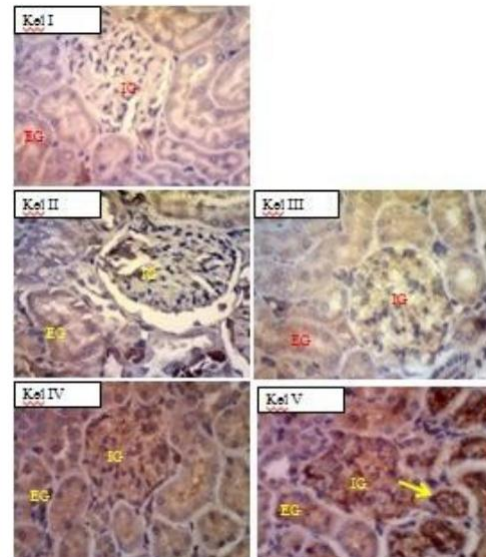


Figure 1: Microscopic picture of kidney slide in 400 $\times$ magnification for group I, II, III, IV and V that have been stained by immunohistochemistry (brown color $\rightarrow$ shows cells express BMP-7 protein in cytoplasm area). IG=intraglomerular, EG=extraglomerular

4. Discussion and Conclusion

Cox et al. (2012) expressed salt can induce fibrosis in the heart, kidneys and cardiovascular system , which was shown in two separate cohort studies in human populations. Yu et al. (1998) also showed that salt induces fibrosis in kidneys, left ventricle and intramyocardial artery of rats. Kidney fibrosis cause end stage renal disease (ESRD) which worsens the condition of kidneys. Induction of fibrosis in the kidney increases blood pressure and induces chronic and acute kidney disease.

In chronic and acute kidney disease, the expression of BMP-7 is decreased; meanwhile TGF- β 1 expression is increased. Physiology restoration of BMP-7 expression in kidney was associated to kidney structure regeneration. Herefore, TGF- β 1 plays a role as pathogenic molecule; meanwhile BMP-7 can be protective agent (Zeisberg et al., 2004).

TGF- β 1 signaling is affected by Phosphorylation-Smad2 that be induced by the binding between TGF- β 1 and its receptor. In contrast, Smad6 and bone morphogenetic protein receptor type-I (BMPR-1) prevent phosphorylation-Smad2 and cause disintegration of Smad2 complex (Zhang et al.,2014). In addition, Zhong et al. (2013) explained that rhBMP-7 can stop Smad-2/-3 nuclear translocation in primary hepatic stellate cells (PHSCs) and hepatocyte, so that liver fibrosis doesn't be formed [25]. Smad-2/-3 nuclear translocation mechanism in liver and kidney are activated by TGF- β 1. Herefore, the increase of BMP-7 expression prevents kidney fibrosis with disintegration of Smad2 complex and stopping of Smad-2/-3 nuclear translocation mechanism (Goebel et al., 2006).

Finally, telmisartan reduces the expression of TGF- β 1 and increase BMP-7 expression.

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