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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2015.05.007

Protective effect of telmisartan on rats with renal failure and its mechanism

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ARTICLE INFO

Article history:
Received 15 Mar 2015
Received in revised form 20 Apr 2015
Accepted 15 May 2015
Available online 25 June 2015

Keywords:
Telmisartan
5/6 Nephrectomy
Renal failure

ABSTRACT

Objective: To study the protective effect of telmisartan on rats with renal failure and its mechanism.
Methods: 60 Wistar rats were chosen as study objective, and were divided into 4 groups randomly: 15 in group A (sham operation group), 15 in group B (model group), 15 in group C (telmisartan group) and 15 in group D (telmisartan + GW9962 group). The difference of survival rate, blood-urine biochemical indexes, renal pathological change, and the expression level of PPARγ and nNOS were compared.
Results: After 12 weeks, the survival rate of group A was 93.33% (14/15), that of group B was 46.67% (7/15), that of group C was 86.67% (13/15), that of group D was 60.00% (9/15), and the difference among 4 groups had statistical significance ($P < 0.05$). After 1 week, the difference of Scr, that of BUN and that of 24 h protein urine among 4 groups was not statistical significant ($P > 0.05$); after 3 weeks, 6 weeks and 12 weeks, these difference was statistical significant ($P < 0.05$). The difference of blood-urine biochemical indexes, that of renal pathological change, and that of the expression level of PPARγ and nNOS was statistical significant ($P < 0.05$).
Conclusions: Telmisartan has protective effect on renal failure caused by 5/6 nephrectomy, which might be relative to the expression level of PPARγ and nNOS.

1. Introduction

Renal failure refers to partial or complete loss of kidney function which is induced by renal disease or injury developing to the final stage, including acute renal failure and chronic renal failure. Renal failure has the characteristics of high death and disability. The patient's life mainly relies on dialysis which is costly [1,2]. It is found that part of angiotensin receptor blockers have a protective effect on kidney, among which telmisartan as part of the activator of peroxisome proliferator activated receptors γ plays a dual role of interdicting AR and activating PPARγ [3,4]. In the present study, we aimed to explore the protective effect of telmisartan on rats with renal failure and its mechanism.

2. Materials and methods

2.1. Experimental animals and groups

A total of 60 healthy male Wistar rats weighting 200–220 g [average weight (210.4 ± 6.2) g] were chosen as study objective. Rats were provided by Laboratory Animal Center of Tianjin Medical University (certificate of approval no. 14-0812). 60 rats were randomly divided into four groups: group A (sham operation group), group B (model group), group C (telmisartan group) and group D (telmisartan + GW9962 group) with 15 rats in each group.

2.2. Drugs and reagents

2.2.1. Drugs

Telmisartan (Yichang Changjiang Pharmaceutical Co., Ltd, approval no. 20140105) was diluted to the solutions of 0.5 mg/mL and 1 mg/mL with normal saline. GW9662 (Selleck Chemicals, USA, purity > 99.00%) was diluted to the solution of 1 mg/ml with normal saline.

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Peer review under responsibility of Hainan Medical University.
Foundation project: It is supported by Natural Science Foundation of Shandong Province (No. ZR2009CL018).

2.2.2. Reagents

Rabbit anti-rat PPAR gamma antibodies and nNOS antibodies (primary antibodies) were provided by Santa Cruz Biotechnology, Inc. HRP goat anti-rat IgG (secondary antibodies) was provided by Shanghai Xueman Bio-Tech Co., Ltd. Other reagents were provided by Beijing Cell Chip Biotechnology Co. Ltd.

2.3. Experimental method

2.3.1. Model establishment and administration

All the rats were fed 7 days to adapt the environment. 5/6 nephrectomy was performed in rats of group B and D to establish renal failure model. After anesthetization by intraperitoneal injection of 2% pentobarbital sodium (40 mg/kg), rats were fixed on the operating table with prone position, then suitable operating field was selected for skin preparation. After disinfection, skin was cut along the bilateral costalspinal angle. The subcutaneous tissue and muscle were separated and renal capsule was decorticated. Bilateral renal arteriovenous and ureter were ligatured and the right kidney was completely removed. After ligation of higher and lower part of the kidney, 1/3 kidney was cut and left side ligature was loosened. Renal capsule of rats in sham operation group was decorticated without resection of kidney.

In group C, 2 mL telmisartan solution (0.5 mg/mL) was used for gavage once a day at 10: 00 am. Rats in group D were given 1 mL telmisartan (1 mg/mL) and 1 mL GW9662 solution (1 mg/mL) to mix gavage once a day. Rats in groups A and B were given with 2 mL normal saline for gavage once a day for 12 weeks.

2.3.2. Detection of blood and urine biochemical indicator

Fasting tail venous bloods of all rats were obtained and 24-h urine specimens were collected in metabolic cages. SCr, BUN and 24-h proteinuria were detected. The above indexes should be examined in all the feeding rats in 1, 3, 6 and 12 weeks. Meanwhile, the survival rate in each group was recorded at the end of each weekend.

2.3.3. Pathological change of glomerulus

After 12 weeks, all the survival rats were killed by cervical dislocation. The residual kidney tissues of rats were picked with prone position, among which 2/3 were washed with PBS buffer solution to pale and fixed with 10% neutral formalin to prepare for paraffin imbedding. Injury degree of rat kidney tissue was observed under light microscope by Periodic acid-Schiff stain. Raji cell assay was used to calculate the CSI of glomerulus. Part of sections were stained with MASSON and tubule interstitial score (TIS) was evaluated under light microscope.

2.3.4. Detection of PPARγ and nNOS expressions by western blot

Part of renal tissues were cut into pieces and digested with 0.5% pancreatin to prepare single-cell suspension. Then the single-cell suspension was centrifuged at 1 000 r/min for 5 min and washed with PBS, and after lyse cells, total protein was obtained. After protein quantification, SDS-PAGE electrophoresis, transferred to a membrane, sealed, and cut, rabbit anti-rat PPAR gamma and β-actin antibodies were added for incubation at 4 °C overnight. After washing, HRP goat anti-rat IgG (secondary antibody) was added for incubation at room temperature for 1.5 h, and then the membrane was washed. ECL kit was used for exposure and development. Detection of nNOS was same as PPARγ.

2.4. Statistical analysis

Data were analyzed with SPSS15.0 statistical software. Measurement data were expressed as mean ± sd. One-way analysis of variance was used for the analysis of comparison among groups. The blood and urine biochemical indicator of rats at different time was analyzed with repeated measures analysis of variance, and measurement data were compared with χ^2 test. $P < 0.05$ was considered as significantly different.

3. Results

3.1. Survivorship curve of each group

After 12 weeks, survival rate in group A was 93.33% (14/15), group B 46.67% (7/15), group C 86.67% (13/15), and group D 60.00% (9/15). The difference was statistically significant ($P < 0.05$) (Figure 1).

3.2. Blood and urine biochemical indicator in each group

Before operation and after 1 week, SCr, BUN and 24-h proteinuria of each group had no statistical difference ($P > 0.05$), while after 3, 6 and 12 weeks, SCr, BUN and 24-h proteinuria had statistical difference ($P < 0.05$). SCr, BUN and 24-h proteinuria of groups B, C and D in 3, 6 and 12 weeks had statistical significant difference ($P < 0.05$) compared with that before operation (Table 1).

3.3. Histopathological change of rat renal tissue in each group

The glomerulus and renal tubules in rats of group A were roughly normal with occasional thickening of glomerular and renal tubules basement membrane. Lesion of glomerulus and renal tubules in rats of group B were obvious with clear inflammatory cells infiltration and physaliphore. Lesions of glomerular in groups C and D were ligher, and expansion and edema could be seen in renal tubules. The lesion degree of group C was less than that in group D. CIS and TIS in each group had statistical significant difference ($P < 0.05$) (Figure 2, Table 2).

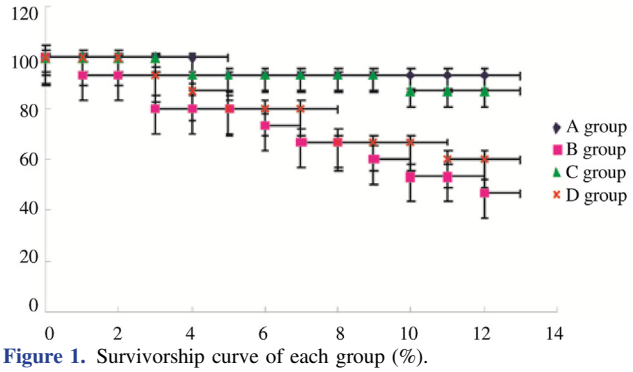

Figure 1. Survivorship curve of each group (%).

Table 1
Blood and urine biochemical indicator in each group (mean ± sd).

Time	Group	Blood and urine biochemical indicator		
		SCr (umol/L)	BUN (mmol/L)	24-h Proteinuria (mg)
Before operation	A Group	32.5 ± 6.4	9.4 ± 1.3	27.8 ± 3.9
	B Group	33.9 ± 6.3	9.1 ± 1.2	27.5 ± 4.1
	C Group	32.7 ± 6.5	9.3 ± 1.4	27.6 ± 3.8
	D Group	32.1 ± 6.6	9.5 ± 1.5	27.7 ± 3.7
1 w	A Group	32.8 ± 6.1	9.5 ± 1.1	28.0 ± 3.5
	B Group	36.8 ± 6.9	11.3 ± 1.5	30.4 ± 4.0
	C Group	32.9 ± 6.4	9.6 ± 1.3	28.4 ± 3.9
	D Group	33.9 ± 6.5	10.4 ± 1.7	28.7 ± 3.8
3 w	A Group	33.1 ± 5.5	9.7 ± 1.4	28.1 ± 3.8
	B Group	59.5 ± 11.8[a]	17.2 ± 2.1[a]	32.4 ± 5.9[a]
	C Group	37.8 ± 5.9[a]	11.7 ± 1.1[a]	29.1 ± 4.3[a]
	D Group	42.6 ± 6.8[a]	14.8 ± 1.9[a]	29.5 ± 4.1[a]
6 w	A Group	34.3 ± 5.9	10.1 ± 1.5	28.6 ± 4.0
	B Group	95.8 ± 14.7[a]	26.5 ± 2.9[a]	45.2 ± 9.1[a]
	C Group	44.9 ± 7.8[a]	14.5 ± 2.0[a]	33.1 ± 4.9[a]
	D Group	54.7 ± 9.5[a]	16.2 ± 2.3[a]	37.4 ± 6.2[a]
12 w	A Group	35.6 ± 7.6	10.5 ± 1.7	29.5 ± 4.2
	B Group	101.7 ± 15.8[a]	27.8 ± 3.1[a]	46.9 ± 9.8[a]
	C Group	47.8 ± 8.2[a]	15.1 ± 2.2[a]	35.4 ± 5.1[a]
	D Group	58.6 ± 10.7[a]	18.9 ± 2.5[a]	40.3 ± 6.9[a]

[a] Compared with that before operation.

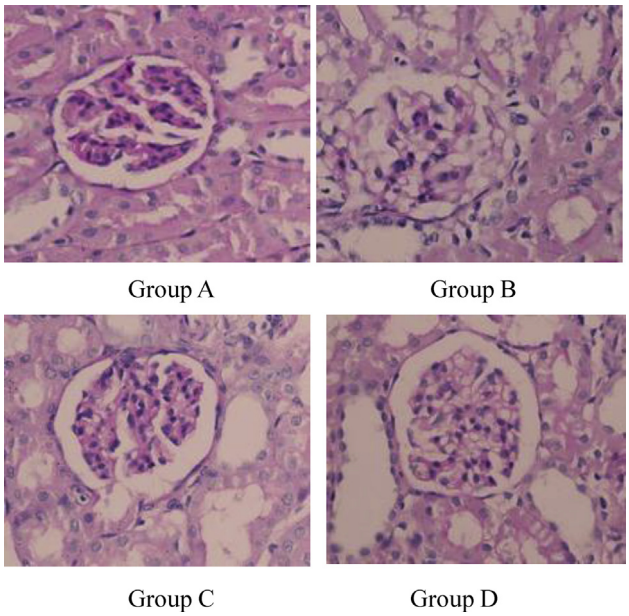

Figure 2. Histopathology of kidney in each group.

Table 2
CIS and TIS of each group (mean ± sd).

Groups	CSI	TIS
A Group	3.44 ± 0.97	0.25 ± 0.11
B Group	21.82 ± 4.75	2.57 ± 0.81
C Group	9.76 ± 1.25	0.77 ± 0.23
D Group	14.53 ± 3.23	1.76 ± 0.54
F	8.097	6.343
P	<0.05	<0.05

3.4. Expression levels of PPARγ and nNOS in each group

Expression levels of PPARγ and nNOS in each group had statistical difference ($P < 0.05$) (Figure 3, Table 3).

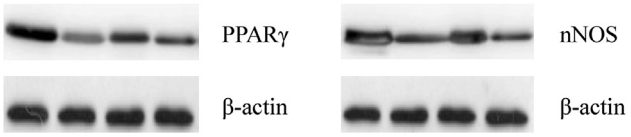

Figure 3. Expression of PPARγ and nNOS in each group.

Table 3
Expression of PPARγ and nNOS in each group (mean ± sd).

Groups	PPARγ	nNOS
A Group	0.86 ± 0.17	0.91 ± 0.19
B Group	0.37 ± 0.09	0.41 ± 0.08
C Group	0.62 ± 0.12	0.65 ± 0.13
D Group	0.41 ± 0.10	0.45 ± 0.11
F	4.231	4.985
P	<0.05	<0.05

4. Discussion

Telmisartan, belonging to ARB drugs, can control blood pressure through inhibiting renin-angiotensin-aldosterone system, which is widely used in the treatment of hypertension [5]. It is found that the structure of telmisartan has differences with other ARB drugs, and it has the activity of PPARγ agonist [6]. PPARγ has extensive biological function and is closely associated with the process of glucolipid metabolism of body, formation of atherosclerotic plaque and the proliferation of vascular smooth muscle, meanwhile, PPARγ plays a role in the progress of glomerulus and renal tubules lesions [7,8]. Moreover, the decrease of expressions of nNOS in renal cortex has a certain correlation with renal injury [9]. In the present study, we established the model of renal failure in rats by 5/6 nephrectomy and studied the protective effect of telmisartan on kidney and the correlation of PPARγ and Nnos.

Renal failure refers to partial or complete loss of kidney function which is induced by renal disease or injury developing to the final stage. Normal kidney has certain compensatory ability, while after 5/6 nephrectomy, kidney function is rapidly declined and presents the state of decompensation. Therefore, 5/6 nephrectomy can used to establish model of renal failure in rats [10]. Rats in each group were performed different operations and given different drugs. After 12 weeks, survival rate of rats in sham operation group was 93.33% (14/15), and rats were unable to tolerate the procedure and died in the operation. Survival rate of rats in model group was 46.67% (7/15), and the cause of death included unable to tolerate the procedure, infection and severe arrhythmia induced by hyperkalemia. Survival rate of rats in telmisartan group was 86.67% (13/15) and rats died of postoperative infection. Survival rate of rats in telmisartan + GW9962 group was 60.00% (9/15) and the cause of death was the same as the model group rats, and the difference was statistically significant. In the detection of blood and urine biochemical indicator in each group, SCr, BUN and 24-h proteinuria of 3, 6 and 12 weeks had statistical differences. Blood and urine biochemical indicator of 3, 6 and 12 weeks in rats with nephrectomy were significantly decreased compared with that before operation. During the observation of histopathological change of rat renal tissue in each group, the glomerulus and renal tubules in rats of sham operation group were roughly normal with occasional thickening of glomerular and renal tubules basement

membrane. Lesion of glomerulus and renal tubules in rats of model group were obvious with clear inflammatory cells infiltration and physaliphore. Lesions of glomerular in telmisartan and telmisartan + GW9962 groups were ligher, and expansion and edema could be seen in renal tubules. The lesion degree of telmisartan group was less than that in telmisartan + GW9962 group. The study showed that after 5/6 nephrectomy, renal injury and renal function of the rats were obviously decreased, and telmisartan could play the protective effect on kidney. When selective PPARγ antagonist GW9662 was combined with telmisartan, the effect of telmisartan declined, thus showing that the protective effect of telmisartan on kidney is independent of RAAS, and is related to PPARγ [11,12].

It was found that the expression levels of PPARγ and nNOS in rats with nephrectomy were significantly decreased compared with that in sham operation group, while the expression levels in telmisartan group were higher than other two groups. It showed that when renal failure occurred, the expression levels of PPARγ and nNOS in rats were decreased, and telmisartan played a protective role in kidney by up-regulating the expression levels of PPARγ and nNOS.

Results showed that the biological activity of PPARγ is wider and can involve in the processes including glucolipid metabolism of the body, inflammatory response, immunologic process and the regulation of cell cycle and apoptosis [13]. It was reported that PPARγ can inhibit glomerular sclerosis, renal interstitial inflammatory cell infiltration and fibrosis through lowering the expression of genes in glomerular, such as PAI, TGF and Type IV collagen, thus relieving renal lesion [14]. Nitric oxide is closely related to the function of glomerular, including expanding afferent glomerular arteriole, regulating haemodynamics, maintaining blood perfusion of the kidney, balancing the feedback effect of glomerular and renal tubules, inhibiting water and sodium reabsorption, and regulating autonomic nerve activity of glomerulus [15,16]. nNOS expression level has obvious relevance with chronic kidney disease progression and the occurrence of kidney failure. The decrease of nNOS in renal cortex is related to the progress of decline in renal function, thickening of glomerular basement membrane and glomerular sclerosis [17].

In conclusion, telmisartan has protective effect on renal failure induced by 5/6 nephrectomy, which may be related to the expression level of PPARγ and nNOS.

Conflict of interest statement

We declare that we have no conflict of interest.

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