Effect of Spironolactone on Hypertrophy of Left Ventricular Myocardium in Wistar Rats with Experimental Uremia

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Chronic renal failure was modeled in rats by partial nephrectomy. Blood pressure, heart rate, concentrations of aldosterone, urea, creatinine, electrolytes, and protein, index of hypertrophy of visceral organs, and 24-h diuresis were evaluated. In rats treated with spironolactone, the index of myocardial hypertrophy did not considerably differ from that in sham-operated animals, whereas in untreated rats the test parameters considerably differ from the control. We concluded that the blockade of aldosterone receptors with spironolactone produced a cardioprotective effect in Wistar rats with subtotal nephrectomy.

Key Words: *aldosterone; spironolactone; arterial hypertension; left-ventricular hypertrophy*

Damage to the cardiovascular system in patients with chronic renal pathologies is frequently diagnosed and considerably affects the prognosis. According to US Renal Data System [11], and Russian Register of Chronic Renal Failure (CRF) [1], cardiovascular complications are the main cause of death in patients with chronic kidney diseases (30-52%). By the moment of the beginning of replacement therapy, more than 50% patients have signs of cardiovascular pathology resulting from hyperproduction of angiotensin II and aldosterone (AS) due to activation of the renin-angiotensin-aldosterone system, hypertension, anemia, hyperlipidemia, atherosclerosis, uremic toxins, medium-molecular weight molecules, secondary hyperparathyrosis determined by disturbances in calcium and phosphorus homeostasis [5,8]. The possibilities of the pathogenetic approach to the correction of cardiovascular damages in chronic renal diseases are not completely realized.

Here we studied the effect of AS on structural changes in the myocardium and the effect of AS receptor blocker spironolactone on hypertrophy of the left ventricle.

MATERIALS AND METHODS

Experiments were carried out on 47 mature male Wistar rats weighing 180-200 g. Subtotal resection of the kidneys ($^5/_6$) was performed, which is a standard method of CRF modeling. The surgery was performed in two stages with 1-week interval under sodium thiopental narcosis. Retrolumbar approach to the kidney was used. During stage 1, resection of $^2/_3$ left kidney was performed as described previously [4]. Before resection, the kidneys were decapsulated for protection of the adrenal glands. The second stage consisted in extirpation of the right kidney. Hemostasis was attained by using a medi-

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cal hemostatic sponge and temporal clumping of the renal artery with a soft clamp; the time of ischemia was 3-5 min. For preventing intraoperation infection and compensation of postoperation dehydratation, the animals received penicillin (1000 U/100 g body weight) and 3 ml NaCl after each stage of the surgery.

On the next day after stage II, the animals were divided into experimental groups. Group 1 animals (n=15) received AS receptor blocker spironolactone (Gedeon Richter) in a dose of 0.2 mg/day with drinking water. For preventrion of hyperkalemia, the animals received furosemide (Aventis Pharma Ltd) in a dose of 0.25 mg/day. Group II (n=5) comprised animals with experimental CRF. Group 3 (controls, n=17) consisted of sham-operated animals. The animals were observed for 10 week after the second stage of surgery. This short period of observation is determined by short life-time (about 2.5-3 years), 10 weeks of rat life correspond to about 7 years of human life.

Before sacrifice, systemic blood pressure (SBP) was measured by the cuff method and HR was determined. Electrogram and cuff pressure curve were recorded using a N-338-2P direct writer (type speed 10 mm/sec). The level of SBP corresponded to cuff pressure at the moment of cessation of pressure pulse. Four to five measurements were performed for each animal.

One day before the end of observation, the rats were placed into a metabolic chamber and fixed for urine collection. The blood was collected during sacrifice. The concentration of AS in the plasma, the concentration of urea, creatinine, potassium, sodium, and total protein in the serum and urine were measured. During evaluation of urine parameters, 24-h diuresis was measured for each rat. The degree of myocardial hypertrophy was evaluated by the index of hypertrophy calculated as the ratio of left ventricular weight to body weight [6,7].

The significance of differences between the groups was evaluated using Student's t test.

RESULTS

In rats of groups 1 and 2, the concentrations of potassium, urea, and creatinine in the blood and urinary excretion of potassium and proteins were higher than in the control group (Table 1, 2). In groups 1 and 2, plasma concentration of AS surpassed the control level, but the difference from the control was more pronounced in group 1.

Index of myocardial hypertrophy in group 1 animals did not significantly differ from that in group 3 (Table 3).

In groups 1 and 2, SBP was significantly higher than in group 3; HR was similar in all groups (Table 3).

The increase in serum contents of urea and creatinine, daily urinary excretion of potassium and protein, and SBP in rats of groups 1 and 2 resulted from the decrease in the number of functioning nephrons and development of CRF against the background subtotal nephrectomy, which agrees with published reports [10].

In group 1 animals, plasma concentration of AS was higher, which resulted from spironolactone treatment. Spironolactone is known to compete with AS for binding with mineralcorticoid receptors, binding of exogenous AS with these receptors leads to suppression of the feedback regulation mechanism and increase in plasma AS concentration [2].

Myocardial hypertrophy developed in animals of all experimental groups. The pathogenesis of myocardial hypertrophy in uremia is poorly understood. At the initial stages of CRF, this hypertrophy probably represents a compensatory reaction to increased afterload to the heart under conditions of developing arterial hypertension. However, with progression of CRF the adaptation mechanisms are transformed into dysadaptation and hypertrophy becomes a leading cause of left-ventricular dysfunction [3].

Arterial hypertension is a very important but not the only cause in the development of myocar-

TABLE 1. Biochemical Parameters of Blood Serum in Rats with CRF ($M\pm m$)

Parameter	Group 1	Group 2	Group 3
AS, pg/ml	281.67±39.02*	207.55±32.86	145.42±17.41
Potassium, mmol/liter	7.91±0.30*	7.73±0.20*	4.51±0.22
Sodium, mmol/liter	149.5±1.5	152.3±1.5	149.0±1.6
Urea, mmol/liter	18.6±2.5***	18.7±0.9***	6.1±0.2
Creatinine, mmol/liter	0.070±0.003***	0.070±0.003***	0.040±0.002
Total protein, g/liter	62.9±1.2	62.8±1.8	63.6±1.9

Note. *p<0.05, **p<0.01, ***p<0.05 compared to group 3.

TABLE 2. Biochemical Parameters of Urine in Rats with CRF (*M*±*m*)

Parameter	Group 1	Group 2	Group 3
Potassium, mmol/day	2.02±0.15**	2.10±0.21*	0.92±0.08
Sodium, mmol/day	0.16±0.03	0.24±0.07	0.26±0.07
Urea, mmol/day	5.66±0.90	6.54±0.45	4.40±0.62
Creatinine, mmol/day	0.08±0.01	0.08±0.01	0.08±0.01
Proteinuria, mmol/day	0.04±0.01***	0.07±0.02*	0.010±0.001
24-h diuresis, ml	13.56±1.78	12.16±1.26	3.79±0.36

Note. *p<0.05, **p<0.001, ***p<0.01 compared to group 3.

TABLE 3. Parameters of Hemodynamics and Myocardial Hypertrophy in Rats with CRF (*M*±*m*)

Parameter	Group 1	Group 2	Group 3
SBP, mm Hg	151.3±2.7**	151.4±2.3**	121.15±1.80
HR, min^{-1}	393.8±17.6	369.0±14.1	347.8±10.3
Index of hypertrophy, mg/g	2.52±0.06	2.88±0.11*	2.35±0.09

Note. *p<0.05, **p<0.001 compared to group 3.

dial hypertrophy. Another important factor of hypertrophy and fibrosis of the myocardium, vessels, and kidneys under conditions of CRF is AC acting through the classical mineralocorticoid mechanism [4]. Upon binding with mineralocorticoids, AS triggers a series of pathological processes; the morphological substrate of these processes are inflammation and fibrinoid necrosis of arteries and arterioles, stimulation of the transforming growth factor-β, and accumulation of extracellular matrix. Apoptosis of cardoimyocytes, changes in the capillary bed, inhibition of fibrinolysis system followed by hyperexpression of plasminogen activator inhibitor-1 lead to the development of endothelial dysfunction, hypertrophy and remodeling of the myocardium and vessels [4].

Some pathogenetic aspects of cardiovascular complications in renal pathologies were revised during the last decade. For instance, it was found that not only angiotensin II, but also AS can act as a damaging factor, moreover, AS can produce adverse affect both in combination with II and independently on it [4,9]. The discovery of the pathogenetic role of AS in the development of fibrosis of the heart and vessels prompted studies of the effects of AS receptor agonists on manifestations of heart failure and prophylaxis of cardiovascular complications. However, the mechanisms underlying the pathogenesis of these complications remain unclear and are the objects of experimental studies until now.

Our experiments demonstrated that in the group of animals treated with spironolactone, the index of myocardial hypertrophy did not significantly differ from that in the control group Our findings agree with published data and confirm that the blockade of mineralocorticoid receptors reduced the degree of left-ventricular hypertrophy and, hence, the risk of cardiovascular complications [9,12].

Thus, AS is an important factor in the development and progression of myocardial hypertrophy in CRF, while blockade of AS receptors with spironolactone in Wistar rats after subtotal nephrectomy produces a cardioprotective effect

REFERENCES

1. B. T. Bikbov and N. A. Tomilina, *Nefrol. and Dializ*, **4**, No. 3, 148-170 (2002).
2. C. S. Barr, C. C. Lang, J. Hanson, *et al., Am. J. Cardiol*., **76**, No. 17, 1259-1265 (1995).
3. M. Epstein, *J. R. Soc. Med.,* **94**, No. 8, 378-383 (2001).
4. *G. M. London, Semin. Dial*., **16**, No. 2, 85-94 (2003).
5. A. C. McMahon, S. E. Greenwald, S. M. Dodd, *et al., Nephr. Dial. Transplant*., **17**, No. 5, 759-764 (2002).
6. K. Okoshi, H. B. Ribeiro, M. P. Okoshi, *et al., Jpn. Heart. J*., **45**, No. 4, 647-656 (2004).
7. M. Sevilla, F. Voces, R. Carron, *et al., Life Sci*., **75**, No. 7, 881-891 (2004).
8. S. Ringoir, *Kidney Int. Suppl.*, **62**, No. 4, S2-S4 (1997).
9. R. Rocha, C. T. Yr. Stier, I. Kifor, *et al., Endocrinology*, **141**, No. 10, 3871-3878 (2000).
10. R. Rocha, C. L. Martin-Berger, P. Yang, *et al., Ibid*., **143**, No. 2, 4828-4836 (2002).
11. *US Renal Data System: USRDS 1999 Annual Data Report National Institute of Health, National Institute of Diabetes and Digestive and Kidney Disease*, Bethesda (1999).
12. K. T. Weber, *Circulation*, **102**, No. 12, 1342-1345 (2000).