Content of Total Homocysteine and Major Aminothiols in Rats with Experimental Renal Ischemia

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The contents of total homocysteine, cysteine, and glutathione in blood plasma and tissue of rats with renal ischemia were measured by HPLC. Our study was performed on the "two-kidney, one-clip (0.13 mm)" model. The concentrations of homocysteine and cysteine in blood plasma from treated rats were higher than in sham-operated animals (control; by 36 and 14%, respectively). Homocysteine level in the intact and clipped kidneys of treated rats was 40% higher than in the control. However, no differences were found in homocysteine level in the ischemic and intact kidneys of treated animals. Cysteine concentration in the clipped kidney was lower than in the kidneys of intact and sham-operated animals (by 1.6 and 1.5 times, respectively). Glutathione concentration in the ischemic kidney did not differ from the control. No differences were revealed in the content of aminothiols in liver samples from rats of the treatment and control groups. Our results suggest that functional inactivation of one kidney is accompanied by impairment of homocysteine catabolism (trans-sulfonation).

Key Words: *hyperhomocysteinemia; cysteine; glutathione; experimental ischemia; kidneys*

Studies over the past decade showed that increased blood level of homocysteine (Hcy) serves as an independent pathogenetic factor for cardiovascular diseases [4,5,6].

In addition to the liver, the kidneys metabolize a considerable amount of Hcy formed via remethylation and cysteine metabolism. Excessive intake of essential amino acid methionine is accompanied by increased formation of Hcy and cysteine (Cys) from methionine. Aminothiol Hcy is an intermediate metabolite of transmethylation reactions in body tissues. Hcy cannot be completely remethylated in tissues. This process is accomplished in the liver and kidneys. An additional cystathionine pathway of these reactions is followed by the formation of Cys. Taurine and sulfates are formed from Cys. Hcy in high concentrations plays an important role in unfavorable remodeling of the vascular wall [2].

Little is known about the role of the kidneys (hypofunction of one kidney) in hyperhomocysteinemia. Renal ischemia results in the development of arterial hypertension. There are no data on the role of renal ischemia in metabolic disturbances of sulfur-containing amino acids. They are manifested in hyperhomocysteinemia, which serves as an independent (non-lipid) factor of atherogenesis.

Here we studied pathogenesis of persistent hyperhomocysteinemia and imbalance of major aminothiols in tissue and blood from rats with ischemia of one kidney.

MATERIALS AND METHODS

Experiments were conducted on male Wistar rats weighing 200-250 g. The animals had free access to water and food. Our study was performed on the "two-kidney, one-clip (0.13 mm)" model (Kent Scientific Corpora-

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tion). The control group consisted of sham-operated rats. Clipping of the right renal artery and sham operation were performed by the standard method [3].

Systolic blood pressure in the caudal artery of awake rats was measured weekly for 2 months. The measurements were performed on a noninvasive blood pressure (NIBP) system (AD Instruments Pty Ltd) with Chart software. Direct measurement of the mean blood pressure in freely-moving animals was conducted after 2 months using an automatic device and Kardio plus software [1]. The kidneys, liver, and blood (separation of the plasma with sodium citrate as a stabilizing agent) were taken from rats under ether anesthesia and stored in a freezing chamber at -80°C. Biochemical studies were performed with blood plasma and 10% homogenates of organs in 0.154 M KCl. The contents of total Hcy (tHcy), Cys, and glutathione (Glu) were measured by HPLC on an Agilent-1100 chromatograph [7]. The results were analyzed by SPSS 17.0 for Windows software.

RESULTS

The contents of tHcy, Cys, and Glu practically did not differ in the right and left kidneys of sham-operated rats (Table 1). The data on these parameters were combined.

No differences were found in aminothiol content in liver samples from animals of the treatment and control groups.

The concentrations of tHcy and Cys in blood plasma from treated rats were higher than in sham-operated animals (by 36 and 14%, respectively; Table 2). No differences were revealed in plasma Glu level in rats of these groups.

The degree of hypertension in ischemic rats was maximum by the 4th week after clipping. The mean systolic pressure in treated animals was elevated to 172.1±20.0 mm Hg (*vs*. 119.60±7.54 mm Hg in the control). However, no correlation was found between blood pressure and aminothiol content in the kidneys and plasma.

tHcy content in intact and clipped kidneys of treated rats was 40% higher than in the control (Table 2). However, no differences were found in tHcy level in the ischemic and intact kidneys of treated animals.

Cys concentration in the intact kidney of treated rats practically did not differ from that in control animals. However, the amount of Cys in the clipped kidney of treated rats was lower than in the kidneys of intact and control animals (by 1.6 and 1.5 times, respectively; $p<0.05$).

Despite the decrease in the concentration of Cys (precursor of Glu) in treated animals, the content of Glu in the ischemic kidney did not differ from the control. No significant differences were revealed in Glu level in the intact kidney of treated rats and kidneys of sham-operated animals.

Spearman correlation test ($r=0.865$) revealed a significant ($p<0.01$) interrelation between the concentrations of tHcy and Cys in the kidneys of sham-operated rats. Such relationships were not revealed in intact and clipped kidneys of treated animals. It should be emphasized that 80% Hcy are degraded (trans-sulfonated)

TABLE 1. Content of tHcy, Cys, and Glu in the Left and Right Kidneys (μmol/g tissue) and Blood Plasma (μmol/liter) of Sham-Operated Rats

Organ/tissue	tHcy	Cys	Glu
Right kidney (*n*=8)	0.091±0.009	4.10±0.04	0.049±0.006
Left kidney (*n*=8)	0.082±0.010	4.00±0.12	0.050±0.004
Total content in the kidneys (*n*=16)	0.087±0.008	4.09±0.06	0.050±0.003
Blood plasma (*n*=8)	4.7±0.9	192.4±6.3	44.6±4.9

TABLE 2. Content of tHcy, Cys, and Glu in the Clipped and Intact Kidneys (μmol/g tissue) and Blood Plasma (μmol/liter) of Treated Rats

Organ/tissue	tHcy	Cys	Glu
Clipped kidney (*n*=11)	0.1270±0.0039*	2.72±0.42^{+++}	0.049±0.006
Intact kidney (*n*=11)	0.1160±0.0028*	4.57±0.35	0.047±0.005
Blood plasma (*n*=11)	6.39±0.29*	219.6±11.8*	42.0±4.2

Note. *$p<0.05$ compared to the control (sham-operated animals); $^{+++}p<0.001$ compared to the intact kidney.

in the kidneys with the formation of cystathionine and Cys. The absence of significant correlations between tHcy and Cys in the kidneys of treated rats probably reflects impairment of this pathway of Hcy catabolism. Two-tailed Spearman correlation coefficient between the concentrations of tHcy in blood plasma and intact kidney of treated animals was high (r=0.758), which indirectly confirms our assumption.

Clinical trials showed that renal dysfunction (*e.g.*, decrease in glomerular filtration) is accompanied by an increase in blood tHcy concentration [5]. Moreover, the tubular apparatus is the major site of Hcy metabolism in the kidneys [2].

Our results indicate that functional inactivation of one kidney can serve as a pathogenetic factor for moderate hyperhomocysteinemia. Experimental observations showed that persistent hyperhomocysteinemia is observed 2 months after unilateral renal ischemia.

This experimental approach can be used for modeling not only experimental hypertension, but also moderate hyperhomocysteinemia. The increase in Hcy concentration contributes to progressive atherosclerosis in humans. Metabolic disturbances of Hcy during renal ischemia probably serve as a major cause of atherosclerotic remodeling in the vascular wall.

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