Effect of Uridine on Energy Metabolism, LPO, and Antioxidant System in the Myocardium under Conditions of Acute Coronary Insufficiency

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Experiments on rats have shown that preventive treatment with uridine stabilizes energy metabolism in the heart under conditions of 60-min left coronary artery occlusion. The preparation also prevented antioxidant system dysfunction and LPO hyperactivation. 5-Hydroxydecanoate, a selective blocker of mitochondrial ATP-dependent K^+-channels, abolished the protective effect of uridine, which attested to the involvement of these channels into mechanisms of the cardioprotective effect of the preparation. The elimination of intravenously administered uridine from the blood of animals with acute ischemia was accelerated in comparison with that in intact animals, which could suggest the participation of this nucleoside in the processes of activation of intracellular anti-ischemic defense mechanisms.

Key Words: *myocardial ischemia; mitochondrial ATP-dependent K^+-channels; energy metabolism; uridine; 5-hydroxydecanoate*

Activation of endogenous protective mechanisms preventing or limiting the ischemic or reperfusion myocardial injuries is the basis of the new strategy in the therapy and prevention of CHD [11]. A prospective approach in this field is evaluation of the possibility of using natural metabolites as the pharmacological preparations preventing the development of energy instability, morphofunctional abnormalities, and cell death caused by disturbed blood supply to the myocardium. The molecular mechanism of cardiomyocyte protection in this case in realized via activation of a multicomponent system including triggers, receptors, and transmitters. The final effector elements in the chain of events leading to ischemic tolerance are believed to be mitochondrial ATP-sensitive K^+-channels (mitoK_{ATP}-channels) [7]. It was previously demonstrated that uridine-5'-diphosphate acts as a direct activator of these channels [5,10]. However, the use of this compound for the pharmacological regulator of mitoK_{ATP}-channels is hampered by its instability and inability to cross the cell membranes. At the same time, low-toxic and membrane-permeable uridine can supply the intracellular pool of uridine-5'-diphosphate in cardiomyocytes [6]. Our previous experiments have shown that uridine reduces the size of the ischemic focus at the early term of acute myocardium infarction (AMI) [9]. This effect is blocked by 5-hydroxydecanoate (5-HD), a selective inhibitor of mitoK_{ATP}-channels [5,8].

Here we studied the effect of uridine on energy metabolism, LPO, and antioxidant system (AOS) in the myocardium under conditions of acute coronary insufficiency and the role of mitoK_{ATP}-channels in the realization of its effects.

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MATERIALS AND METHODS

The experiments were carried out on 150 male Wistar rats weighing 250-300 g. We performed two experimental series. In series I, the cardioprotective effect of uridine was examined. The animals were divided into 4 groups: intact rats (group 1), control rats with AMI (group 2), rats with AMI pretreated with uridine (group 3), and rats with AMI receiving 5-HD prior to uridine.

The animals were narcotized with sodium ethaminal (50 mg/kg). Myocardial ischemia was modeled by ligation of the descending branch of the left coronary artery (LCA) at the level of the lower edge of the left auricle under conditions of jet ventilation. Uridine (30 mg/kg) was injected intravenously 5 min before LCA occlusion and 5-HD (5 mg/kg) was injected intravenously 5 min before uridine. Both agents were dissolved in physiological saline. The controls received the same volume of the solvent.

In 60 min after LCA occlusion, the content of ATP (by HPLC) [4], creatine phosphate, lipid peroxides, and reduced glutathione and SOD activity were measured [1,3]. Serum paraoxonase was assayed [9].

In experimental series II, the rate of uridine elimination from the blood after its intravenous injection was evaluated by HPLC. The animals were divided into 3 groups: intact rats (group 1), intact rats to whom 30 mg/kg uridine was injected intravenously and its blood concentration was measured 5 min postinjection (group 2), and rats with AMI (control, group 3) receiving intravenous injection of 30 mg/kg uridine 5 min before LCA occlusion; uridine concentration in these rats was measured immediately after occlusion. Blood samples for measurement of uridine concentration were then taken 5, 10, 15, 30, and 60 min after LCA ligation. The blood uridine content in intact animals was taken as the natural background.

Significance of differences was evaluated using nonparametric Mann–Whitney test and Student *t* test.

RESULTS

Occlusion of LCA for 60 min reduced ATP content in the rat heart by 35% in comparison with that in intact animals (Table 1). The content of another macroergic compound creatine phosphate also considerably decreased by 59%. The deficiency of macroergic compounds in the ischemic myocardium was accompanied by pronounced accumulation of lipid peroxides (by 97%) and suppression of SOD activity (by 28%) and glutathione system, which resulted in a decreased content of reduced glutathione (by 30%). Serum paraoxonase activity increased by 93%. This HDL-associated enzyme exhibits antiatherogenic and antioxidant properties and prevents lipid oxidation in HDL [12].

The administration of uridine 5 min prior to LCA occlusion limits changes of energy metabolism in ischemic myocardium and preserved high content of ATP and creatine phosphate. Moreover, uridine prevented overproduction of lipid hydroperoxides and inhibition of AOS enzymes in animals of this group. These findings attest to preserved balance between LPO processes and AOS activity that is essential for intracellular homeostasis.

5-HD administered 5 min before uridine injection and subsequent LCA occlusion abolished the protective effect of this preparation. In animals of this group, the content of ATP and creatine phosphate remained at the control level. Prevention of lipid hydroperoxide overproduction and AOS activation by uridine were also abolished after blockade of mitoK_{ATP}-channels. These findings suggest that the energy-stabilizing effect of uridine and its capacity to prevent LPO intensification and AOS inhibition are related to activation of mitoK_{ATP}-channels.

TABLE 1. Effect of Uridine and Combination of 5-HD+Uridine on Parameters of Energy Metabolism, LPO, and Antioxidant System in AMI Caused by 60-min LCA Occlusion (*M*±*m*; *n*=8-10)

Group	Myocardium					Blood serum
	ATP, μmol/g	creatine phosphate, μmol/g	lipid hydroperoxides, OD_{480}	SOD, arb. units/mg protein	reduced glutathione, μmol/g	paraoxonase, μmol/min/liter
Intact	2.54±0.15	6.63±0.18	0.070±0.003	2.27±0.02	34.37±0.62	21.39±1.69
AMI (control)	1.65±0.15*	2.75±0.13*	0.138±0.014*	1.63±0.01*	23.99±1.02*	41.40±3.39*
Uridine+AMI	2.81±0.13$^+$	6.56±0.26$^+$	0.077±0.003$^+$	1.99±0.05$^+$	33.61±1.73$^+$	26.47±1.97$^+$
5-HD+uridine+AMI	1.41±0.05*	2.89±0.29*	0.124±0.005*	1.65±0.04*	20.75±1.25*	43.56±23.21*

Note. *$p<0.05$ in comparison with intact animals; $^+p<0.05$ in comparison with the control.

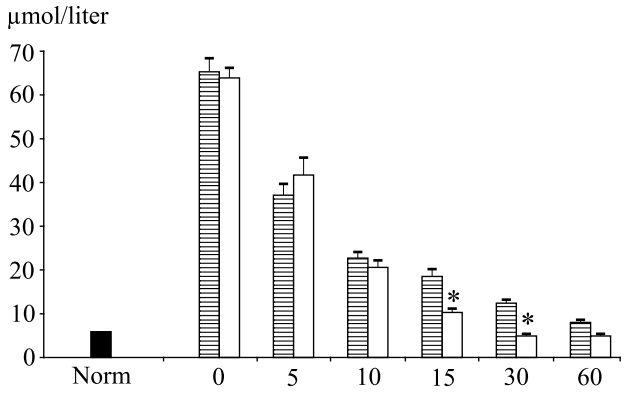

Fig. 1. Effect of AMI on the rate of uridine elimination from circulation after its intravenous injection. Time "0" corresponds to uridine concentration measured 5 min after its injection and the moment of LCA occlusion. Hatched bars: intact rats receiving uridine; open bars: rats with AMI pretreated with uridine. *p<0.05 in comparison with intact rats receiving uridine.

In 5 min after administration of the nucleoside to intact rats, its blood content increased by 11 times in comparison with the baseline value (65.3±3.6 *vs.* 5.9±0.3 μmol/liter; Fig. 1). Similar increase was observed in animals receiving uridine injection before LCA occlusion. The sharp increase in uridine concentration was followed by its elimination. The rate of uridine elimination from the blood was similar in animals with and without LCA occlusion and therefore its concentration did not differ in these groups. However, uridine content in rats with AMI on minute 15 after its intravenous injection sharply decreased to 10.3±0.9 μmol/liter and even dropped below the baseline level by the 30th minute (4.9±0.5 μmol/liter). In 15 min after uridine injection, the blood uridine content in intact rats was 18.5±1.7 μmol/liter, *i.e.* 2-fold lower than in rats with AMI (p<0.05); by the 30th minute this parameter decreased only to 12.4±0.8 μmol/liter. Excessive concentrations of uridine in these animals (8.0±0.6 μmol/liter) persisted over 60 min.

We have previously reported that considerable disturbances of energy metabolism in ischemic myocardium developed as soon as 15 min after LCA ligation and peaked after 30 min [2]. According to our findings, this coincided with accelerated elimination of uridine from the circulation. This is probably related to increased myocardial demands for uridine under hypoxic conditions for activation of intracellular defense and adaptation mechanisms, especially during periods of more pronounced energy metabolism disturbances.

Thus, our experiments have shown that uridine can increase myocardial resistance to oxygen deficiency under conditions of AMI. In animals with myocardial ischemia, this nucleoside rapidly disappears from the circulation and apparently promotes activation of anti-ischemic cardiomyocyte defense mechanisms, which leads to stabilization of energy metabolism and maintenance of the balance between LPO and AOS. The results of the experiments with selective mitoK_{ATP}-channel blocker 5-HD suggest that realization of the protective effect of uridine is related to activity of these channels. The results of this and previous studies drove us to a conclusion that uridine can be used as promising cardioprotective preparation for prevention and therapy of AMI.

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