The Effects of Ulinastatin on Cardiac and Hepatic Energy Metabolism in Rats Subjected to Hypovolemic Shock

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Ulinastatin is a trypsin inhibitor extracted from human urine. In this study the effects of ulinastatin on myocardial and hepatic tissue concentrations of creatine phosphate (CP), ATP, ADP, AMP, lactate, pyruvate, and glycogen have been investigated in rats which were in hemorrhagic shock state. Hypovolemia was induced by bleeding from the femoral artery, and systolic blood pressure was maintained 40 mmHg for 25 min, then ulinastatin 50,000 units·kg^{-1} in saline or saline vehicle was intravenously administered. Thereafter the heart and liver were extirpated and frozen quickly with liquid nitrogen. The tissue concentrations of CP, ATP, ADP, AMP, lactate and glycogen were measured enzymatically. Systolic blood pressure elevated significantly after ulinastatin administration. The myocardial tissue CP level was higher in ulinastatin-treated group than that of control group, whereas no significant difference in energy charge between two groups. The hepatic tissue level of AMP, lactate and L/P ratio was lower in ulinastatin-treated group than that of control group, however, no significant difference was found in hepatic tissue level of ATP, ADP and energy charge. From these results it is concluded that ulinastatin can improve the energy metabolism of myocardium to some extent, but not of the liver in rats with hypovolemic shock. (Key words: Heart, Liver, Metabolism, Hypovolemic Shock, Ulinastatin)

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Ulinastatin, a trypsin inhibitor found in human urine[1], has been identified as an acid glycoprotein with a molecular weight of 67000–68000 containing 5–12% of neutral sugar[2]. The protective effects of ulinastatin in experimental shock have been reported[3].

In this study, the effects of ulinastatin on blood gases, heart rate, systolic blood pressure and tissue metabolites in the myocardium and liver were investigated in hemorrhagic hypotensive rats.

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Material and Methods

Fifteen male Wistar rats weighing between 400 to 500 g were selected. Pentobarbital sodium (50 mg·kg^{-1}) was intraperitoneally administered. The tracheostomy was performed, and intermittent positive pressure ventilation was provided to keep Pa_{CO_2} 35–45 mmHg. Electrocardiograms were recorded with a Nihonkohden's bioelectric amplifier AB-621G. A Millar's microtip catheter pressure transducer (SPR-249) was inserted into the right cervical artery to measure arterial pressure. Hypotension was induced by bleeding from the femoral artery, and sys-

Table 1. Blood gas analysis

	before		25 min	
	ulinastatin (n=6)	control (n=9)	ulinastatin (n=6)	control (n=9)
pH	7.36 ± 0.03	7.33 ± 0.06	7.20 ± 0.05**	7.21 ± 0.06*
P_{O_2}	76.7 ± 18.6	75.1 ± 16.5	80.5 ± 16.7#	114 ± 20*
P_{CO_2}	38.6 ± 1.6	46.1 ± 9.8	40.3 ± 7.8	37.3 ± 6.3***
base excess	−2.4 ± 1.1	−1.2 ± 2.7	−10.6 ± 2.4*	−11.2 ± 2.8*
hematocrit	44.6 ± 2.5	44.5 ± 4.5	37.0 ± 5.6***	37.0 ± 5.1***

#$P < 0.005$ vs control (non-paired t-test)

*$P < 0.001$, **$P < 0.01$, ***$P < 0.02$, compared with the values before hemorrhage (paired t-test).

Table 2. Heart rate and systolic blood pressure

	before	0	5	10	15	20	25	30 (min)
Heart rates (beats·min^{-1})								
ulinastatin (n=6)	293 ± 56	264 ± 43	237 ± 51	243 ± 41	241 ± 37	251 ± 29	255 ± 24	261 ± 26
control (n=9)	324 ± 42	271 ± 35	247 ± 30	244 ± 20	243 ± 23	243 ± 32	238 ± 23	246 ± 28
SBP (mmHg)								
ulinastatin (n=6)	98 ± 21	40 ± 5	39 ± 2	40 ± 2	38 ± 1	39 ± 1	36 ± 2	55 ± 8*
control (n=9)	103 ± 26	42 ± 6	38 ± 4	38 ± 2	39 ± 2	39 ± 3	39 ± 4	41 ± 4

*$P < 0.005$ vs control (non-paired t test)

Ulinastatin or saline vehicle was given at the time of 25 min.

tolic blood pressure (SBP) was maintained 40 mmHg for 25 min with additional bleeding, if it was indicated. Thereafter rats were given ulinastatin (50,000 units·kg^{-1}) in 0.2 ml saline, or saline vehicle with the same volume intravenously.

Blood gas analysis was made before bleeding and right after the administration of ulinastatin or saline. Thoracotomy and laparotomy were performed five minutes after the administration of ulinastatin or saline, and heart and liver were extirpated and frozen rapidly between pre-cooled Wollenberger tongs, and submerged in liquid nitrogen. The tissue specimens were freeze-dried and stored until analysis. The tissue metabolites were extracted from specimens with perchloric acid. Creatine phosphate (CP), ATP, ADP, AMP, lactate (L) and pyruvate (P) were measured enzymatically[4]. Tissue glycogen was also measured as glucose equivalent after digestion of specimen in 30% KOH in a boiling water bath[5]. The data were expressed as micromoles per gram of dry tissue weight, and shown mean ± SD.

The paired and non-paired t test were utilized for statistical significance. Differences were considered significant when $P < 0.05$.

Results

The results of arterial gas analysis are summarized in table 1. Metabolic acidosis and lowered hematocrit were found in both groups at 25 min of hypotension. No differences in pH, P_{CO_2}, base excess and hematocrit were found between the two groups. P_{O_2} increased significantly at 25 min of hypotension in the control group, however, no change was seen in the ulinastatin-treated group.

Table 3. Cardiac tissue concentration of metabolites

	ulinastatin (n=6)	control (n=9)
ATP*	18.08 ± 0.67	16.77 ± 1.68
ADP*	3.37 ± 0.21	3.56 ± 0.93
AMP*	0.402 ± 0.108	0.425 ± 0.376
EC	0.905 ± 0.008	0.894 ± 0.032
CP*	27.36 ± 2.05$^{\#}$	21.80 ± 5.59
pyruvate*	0.77 ± 0.30	0.87 ± 0.21
lactate*	29.99 ± 3.06	35.00 ± 5.85
L/P	42.71 ± 12.65	42.73 ± 14.16
glycogen*	144.1 ± 20.0	143.3 ± 14.2

*μ mole·g^{-1} dry tissue
$^{\#}P < 0.05$ vs control (non-paired t test)

Table 4. Hepatic tissue concentration of metabolites

	ulinastatin (n=6)	control (n=5)
ATP*	4.14 ± 0.54	4.02 ± 1.33
ADP*	4.70 ± 0.65	4.21 ± 0.94
AMP*	0.532 ± 0.090^{+}	0.905 ± 0.366
EC	0.696 ± 0.019	0.666 ± 0.047
pyruvate*	1.34 ± 0.28	1.29 ± 0.44
lactate*	33.71 ± 2.94^{+++}	46.93 ± 4.58
L/P	25.91 ± 4.27^{++}	43.45 ± 12.83
glycogen*	481.9 ± 97.5	310.8 ± 141.8

*μ mole·g^{-1} dry tissue
$^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.005$ vs control (non-paired t test)

Heart rate decreased gradually over 30 min of hypotension in both groups. SBP significantly increased 5 min after the administration of ulinastatin (table 2). The cardiac tissue contents of metabolites are shown in table 3. Except the myocardial CP, which was significantly higher in the ulinastatin-treated group, no significant differences in other tissue metabolites were found between the two groups. In hepatic tissue, AMP, lactate, and the L/P ratio became lower in the ulinastatin-treated group compared with those of control (table 4).

Discussion

Ulinastatin was extracted and purified from fresh human urine by the method of Proksh and Routh[1]. It is known as an acid glycoprotein (molecular weight of 67000–68000) with an inhibiting action to trypsin, α-chymotrypsin, lipase, amylase, elastase and carboxy-peptidase[2]. It is reported that the activity of these enzymes in blood are markedly increased in the patient with acute pancreatitis. Ulinastatin, which inhibits all of these enzymes, seems to be useful for the treatment of acute pancreatitis[6]. Ulinastatin has also been reported to possibly improve metabolic alteration caused by circulatory insufficiency in canine hemorrhagic shock[3].

In this study the effects of ulinastatin on cardiac and hepatic energy metabolism have been investigated in rats in hemorrhagic hypotension.

The arterial gas analysis revealed virtually the same results between the two groups, except P_{O_2}, which was higher at 25 min of hypotension in the control group, due to unidentified cause. The base excess and hematocrit decreased over 25 min of hypotension in both groups. These findings indicated that rats of both groups were in an identical shock state.

The cardiac tissue level of CP remained higher in the ulinastatin-treated group than that of control group, however, no significant difference in ATP, ADP, and AMP contents was found between the two groups. The calculated energy charge in the myocardium was also not significantly different. The term "energy charge" was first coined by Atkinson[7]. The expression takes into account the fact that ATP contains two high-energy phosphate(~P) groups, while ADP contributes one (through the action of adenylate kinase). All three adenine nucleotides strongly influence many reactions that regulate both production and utilization of energy within cell. Due to this, the system counteracts changes in energy charge, which is thus strongly poised. It would seem that the energy charge is a convenient measure of the balance between production and utilization of energy.

Under physiological conditions, myocardial organic phosphates such as CP permit ATP concentration to be maintained while ATP is rapidly being utilized as an energy

source for cell contraction. When ATP is plentiful, its concentration can build up sufficiently to cause the reverse reaction to occur, and allow the concentration of CP to increase substantially so as to act as a store of high energy phosphate. Aoki et al.[8] examined the myocardial CP content in dog, whose coronary artery was ligated for certain period and reperfused. The CP content in the ulinastatin-treated group was higher than that in the untreated control group. From these results it is revealed that the balance between production and utilization of energy in the myocardium was shifted into positive side with ulinastatin treatment, probably because of improved energy production and/or decreased energy utilization.

Several investigators have reported the decrease in hepatic tissue high energy phosphate concentration in shock animals[9-11]. Ohnishi et al.[3] reported that the treatment with ulinastatin for traumatic shock rats, reversed the decreased hepatic ATP level. In this study, there were no significant differences in ATP, ADP and energy charge between the two groups. However, the hepatic lactate content and L/P ratio in the ulinastatin-treated group were significantly lower than those in the control group.

As shown in following equation (a), lactate formation occurs when H is transfered to pyruvate from NADH.

$$\text{Lactate} + \text{NAD}^+ \overset{\text{LDH}}{\rightleftharpoons} \text{pyruvate} + \text{NADH} \quad \ldots\ldots\ldots\ldots \text{(a)}$$

This reaction favors lactate formation so that under normal conditions the pyruvate level is only about 1/10 to 1/20 of the lactate level. There are two NAD^+-NADH systems in the cell, one is in cytoplasm and the other in mitochondrial compartment. The enzyme LDH is exclusively present in cytoplasm, so the lactate formation is not only regulated by the pyruvate concentration but also by the NAD^+/NADH ratio in cytoplasm. The regulation links between NAD^+/NADH ratio and the phosphorylation state of the adenine nucleotides is provided by the following reactions in the glycolytic pathway:

Glyceraldehyde-3-P(GA-3-P) $+ \text{NAD}^+ + \text{Pi} + \text{ADP} \rightleftharpoons$ 3-phosphoglycerate(3-P-G) $+ \text{NADH} + \text{ATP}$

$$\text{sum: } \frac{[\text{NAD}^+]}{[\text{NADH}]} = K \times \frac{[3\text{-P-G}]}{[\text{GA-3-P}]} \times \frac{[\text{ATP}]}{[\text{ADP}][\text{Pi}]}$$

In cytoplasm the existence of a single free pool of nucleotides, with which each of the dehydrogenases is in equilibrium, is suggested. The above equation is rearranged with the LDH reaction as follows:

$$\frac{[\text{NAD}^+]}{[\text{NADH}]} = K_1 \times \frac{[\text{pyruvate}]}{[\text{lactate}]} = K_2 \times \frac{[\text{ATP}]}{[\text{ADP}][\text{Pi}]}$$

This equation shows that a high $[NAD^+]$/[NADH] ratio (or high P/L ratio) will be associated with a high [ATP]/[ADP][Pi] ratio and vice versa[12]. It can be stated that the elevation in L/P ratio frequently observed in the shock state reflects decreased cell phosphorylation rather than the cell hypoxia[13].

In this study, the hepatic high energy phosphate content and energy charge showed no significant difference with ulinastatin treatment, whereas hepatic tissue lactate and L/P ratio were lowered significantly with ulinastatin treatment. We took the total cell ATP, ADP and AMP contents instead of those of cytoplasm for the calculation of phosphorylation states. This can be one reason for explaining the discrepancy between posphorylation state and L/P ratio in hepatic tissue found in this study. With ulinastatin treatment for shock rats, the myocardial CP content was improved, but no significant change was found in phosphorylation state in hepatic tissue. From these results it is concluded that ulinastatin improved the myocardial energy metabolism but not the hepatic energy metabolism in a hemorrhagic shock.

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