The Effects of Urinastatin on the Plasma Levels of Granulocyte Elastase during Open Heart Surgery Under Simple Deep Hypothermia

Takae KAWAMURA, Yoshihiko SHIMODA and Reiji WAKUSAWA

Changes in granulocyte elastase (GLE) and β-gluculonidase (β-gl) were observed during open heart surgeries which were performed under deep hypothermia with surface cooling. In addition, the effect of urinary trypsin inhibitor, urinastatin, on the activities of these enzymes was studied. The patients were divided into three groups, namely group U-I with intravenous injection of 6000 u·kg^{-1} of urinastatin before cooling, group U-II administered with an additional 6000 u·kg^{-1} after warming to 30°C, and an untreated group (Group C). The plasma level of GLE increased significantly in the three groups compared with the level before cooling respectively. In the group U-II, the GLE level after the warming was lower than that in the control group. The serum level of β-gl increased significantly in the three groups at the end of rewarming (36°C). The release of GLE from lysosomes in granulocytes was inhibited in the group U-II. The insufficient inhibition of GLE release in the group U-I is probably due to relatively short half-life of urinastatin. Therefore double administration of 6000 u·kg^{-1}, before and after the cooling, may be required to achieve the therapeutic effect. Consequently, urinastatin appears to be useful in open heart surgery under deep hypothermia with surface cooling. (Key words: hypothermia, cardiosurgery, lysosomal enzyme, protease inhibitor, urinastatin)

(Kawamura T, Shimoda Y, Wakusawa R: The effects of urinastatin on the plasma levels of granulocyte elastase during open heart surgery under simple deep hypothermia. J. Anesth 6: 269–276, 1992)

Simple deep hypothermia can protect many vital organs from deleterious stress during open heart surgery. However the simple deep hypothermia per se may induce peripheral hypoxia due to inadequate peripheral circulation and the increased affinity of hemoglobin for oxygen despite of a suppressed metabolism. Chiba[1] has reported that the serum lysosomal enzymes increase after 60 min of circulatory arrest at the lowest temperature (20°C) period, though hypothermia itself did not effect on the lysosomal membrane. Therefore, in order to prevent postoperative organic disorders it may be worthy to observe changes of humoral mediators which are closely involved in the tissue hypoxia. We measured the plasma levels of granulo-

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Table 1. Case of patient

	patient	age	sex	diagnosis
control	1	11M	M	VSD
	2	6Y	M	ASD
	3	3Y 6M	M	ASD
	4	2Y 3M	M	VSD, PH
	5	6M	M	PS
	6	2Y	M	VSD, PH
	7	1Y 4M	M	VSD, PH
	8	9Y	M	ASD
U-I	1	7Y	F	ASD
	2	3Y 6M	F	ASD
	3	5Y	F	ASD
	4	3Y 5M	M	VSD
	5	9M	M	VSD, PH
	6	2Y	F	VSD
	7	11M	F	LV to RA communication
	8	3Y	M	TOF
U-II	1	5M	F	ASD
	2	2Y	M	VSD
	3	11M	M	VSD, PH
	4	5Y	F	ASD, PAPVR
	5	6Y	F	VSD
	6	1Y	M	VSD, PH
	7	3Y	F	VSD
	8	5Y 6M	M	VSD

VSD: ventricular septal defect, ASD: atrial septal defect, PH: pulmonary hypertension, PS: pulmonary valve stenosis, LV: left ventricle, RA: right atrium, TOF: tetralogy of FALLOT, PAPVR: partial anomalous pulmonary venous return.

cyte elastase which is suspected most likely as an indicator for organ failure, and evaluated the effect of urinastatin, a protease inhibitor purified from human urine, administered during the hypothermia.

Subjects and Methods

The subjects consisted of 24 patients undergoing open heart surgery under simple deep hypothermia (table 1). Patients undergoing emergency operations or those who had been in decompensation due to being severely ill were excluded from the present study. After induction of anesthesia with 5 $mg \cdot kg^{-1}$ thiamylal, their trachea was intubated with the aid of succinyl choline chloride. According to the method of our department[2], following administration of 1.5 $mg \cdot kg^{-1}$ triflupromazine, they were cooled to 20°C by immersion in iced water under deep anesthesia with ether. Prior to the surface cooling, 6000 $u \cdot kg^{-1}$ of urinastatin (Miraclid®) was administered intravenously in eight cases (Group U-I). An identical dose was added while warming at 30°C in eight others (Group U-II). Urinastatin was not used in eight others (Group C). Blood samples were drawn from the radial artery at 6 different periods; before cooling, after cooling to 30°C, at the lowest temperature, after resuscitation, after warming to 30°C, and then after

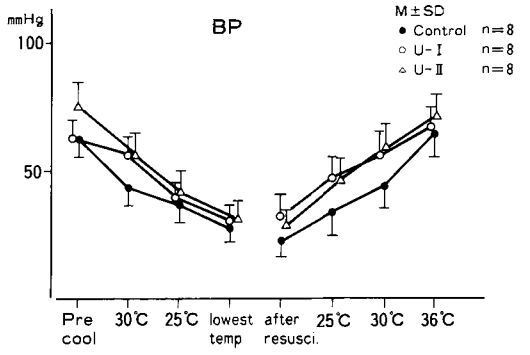

Fig. 1. Mean arterial pressure.
Mean ± SD.
● control (n=8), ○ U-I (n=8), △ U-II (n=8).

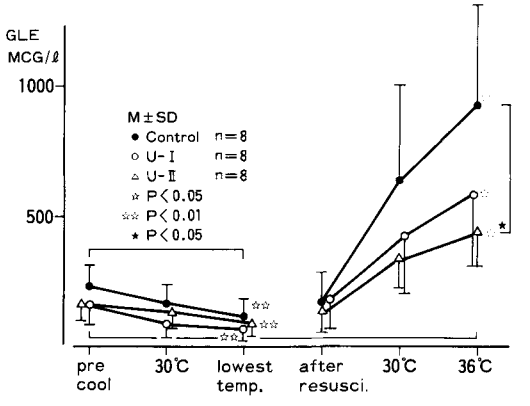

Fig. 2. Mean levels of plasma granulocyte elastase in urinastatin administered and control groups.
Mean ± SD.
● control (n=8), ○ U-I (n=8), △ U-II (n=8).
☆ $P < 0.05$, ☆☆ $P < 0.01$. Significantly different from the period before cooling.
★ $P < 0.05$. Comparison between control and group U-II.

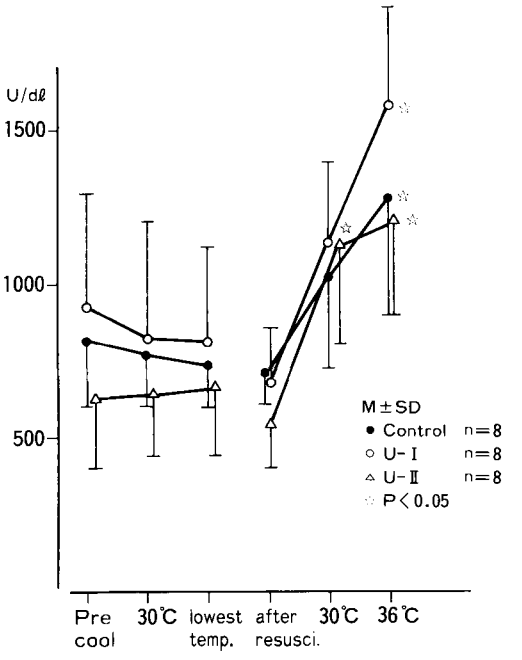

Fig. 3. Mean levels of serum β-glucuronidase in urinastatin administered and control groups.
Mean ± SD.
● control (n=8), ○ U-I (n=8), △ U-II (n=8).
☆ $P < 0.05$. Significantly different from the period before cooling.

completing warming, for measurement of blood levels of GLE and β-gl.

The lowest esophageal temperature around 20 ~ 23°C which was considered as the optimum temperature according to the permissible circulatory arrest time was achieved by cooling. ECG, EEG, arterial pressure (invasive method), esophageal and rectal temperature were monitored. Intravenous drip infusion of lactated Ringer's solution at 3 ~ 5 ml·kg^{-1}·hr^{-1} and 10% low molecular weight dextran solution at 3 ~ 5 ml·kg^{-1}·hr^{-1} was kept throughout.

Immunologic activities of granulocyte elastase and α_1-PI complex were measured by the Sandwich immunoassay method[3] to determine granulocyte elastase concentration. The modified method of Nobunaga[4] was used for the measurement of β-gl. Statistical analysis was performed by using analysis of variance and paired t-test, and $P < 0.05$ was considered significant. All values were expressed as the mean ± SD.

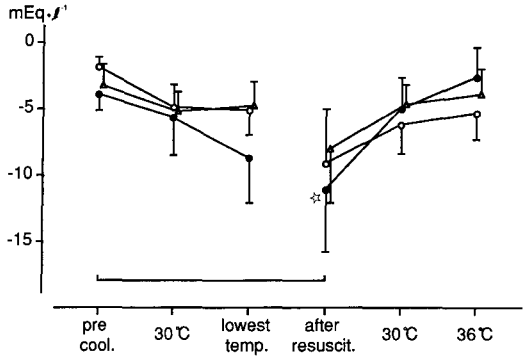

Fig. 4. Base excess during open heart surgery under hypothermia with surface cooling.

Mean ± SD.

● control (n=10), ○ U-I (n=8), △ U-II (n=8).

☆ $P < 0.05$. Significantly different from the period before cooling.

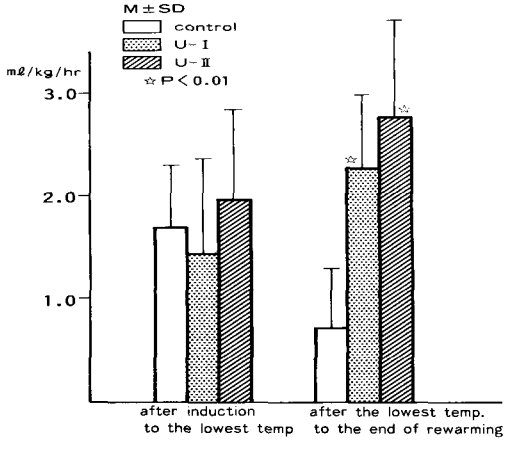

Fig. 5. Mean levels of urine volume in urinastatin administered and control groups.

Mean ± SD.

interval I: interval between induction of anesthesia and maximum cooling.

interval II: interval between maximum cooling and completion of warming.

□ control (n=8) ▨ U-I (n=8)

▨ U-II (n=8)

☆ $P < 0.01$. Significantly different from control group.

Results

(1) Mean blood pressure and heart rate decreased significantly in all three groups of patients following the cooling and increased following the rewarming. No significant differences between the three groups of patients were noted, respectively (fig. 1).

(2) GLE: At the lowest temperature, GLE decreased significantly from 224 ± 71 to 117 ± 54 $\mu g \cdot l^{-1}$ in Group C, from 159 ± 72 to 64 ± 35 $\mu g \cdot l^{-1}$ in Group U-I and from 159 ± 40 to 98 ± 30 $\mu g \cdot l^{-1}$ in Group U-II ($P < 0.01$). GLE began increasing after recirculation, and reached the maximum at the completion of warming (36°C), namely it increased to 861 ± 602 $\mu g \cdot l^{-1}$ in Group C, 597 ± 303 $\mu g \cdot l^{-1}$ in Group U-I and 444 ± 152 $\mu g \cdot l^{-1}$ in Group U-II ($P < 0,01$). These values were far greater than the initial values before cooling, respectively. However, the value of GLE in Group U-II was significantly less than that in Group C ($P < 0.05$) (fig. 2).

(3) β-gl: β-gl began increasing after recirculation in all groups and reached maximum at the completion of warming (36°C). It increased significantly to 1292 ± 367 $u \cdot dl^{-1}$ in Group C, 1599 ± 639 $u \cdot dl^{-1}$ in Group U-I and 1127 ± 291 $u \cdot dl^{-1}$ in Group U-II ($P < 0.05$) (fig. 3).

(4) Base excess in the urinastatin-treated group was compared with that in the untreated group (Group C). There was no significant difference between the level before cooling and after recirculation in Group U-I and Group U-II respectively. However, the base excess in Group C after recirculation was significantly lower than that before cooling ($P < 0.05$) (fig. 4).

(5) Volume of Urine: The volume of urine in the interval between induction of anesthesia and the lowest temperature period was 1.68 ± 2.3 $ml \cdot kg^{-1} \cdot hr^{-1}$ in Group C, 1.44 ±

1.14 $ml \cdot kg^{-1} \cdot hr^{-1}$ in Group U-I, and 1.97 ± 1.4 $ml \cdot kg^{-1} \cdot hr^{-1}$ in Group U-II. No statistically significant difference was noted between the groups. However, between the lowest temperature period and the end of rewarming, it increased significantly to 2.29 ± 0.74 $ml \cdot kg^{-1} \cdot hr^{-1}$ in Group U-I and 2.78 ± 1.2 $ml \cdot kg^{-1} \cdot hr^{-1}$ in Group U-II as compared with that in Group C ($P < 0.01$) (fig. 5).

Discussion

Simple deep hypothermia with surface cooling is also called as "controlled shock," where peripheral and organic microcirculation is impaired. It has been also reported that the serum lysosomal enzymes increase after circulatory arrest[1] under hypothermic anesthesia. In the present study, we measured the changes of granulocyte elastase, which is one of the humoral mediators closely involved in tissue hypoxia. At the same time, we studied the effect of urinastatin, which would have an inhibitory effect on protease activities and would be expected to have antishock effect. Additionally, we studied its effect on β-gl, one of the lysosomal enzymes. GLE is an enzyme released from granulocytic lysosome in patients with such ailments as inflammatory disease[5] or shock[6]. As this enzyme lyzes elastin and fibronectin, it induces degeneration and dissociation of tissues through the progression of such activities. Therefore, it is worthy to predict and to inhibit its elevation in the blood for the prevention of postoperative disorders in many organs. Furthermore GLE has been known to increase during the extracorporeal circulation[7,8] or hemodialysis[9,10]. Its secretory mechanism is considered to be initiated by the activation of complements.

The results of the present study indicated that hypothermic anesthesia itself could stabilize the cellular membrane or inhibit GLE release, since the GLE concentration in the blood was significantly reduced at the lowest temperature as compared with the levels before cooling in the control group as well as in the urinastatin-treated groups. In contrast, the increase in GLE after resuscitation might be attributed to hypoxia during the circulatory arrest or to direct surgical tissue damage. It has been reported that the levels of GLE increases with the onset of myocardial infarction[11], and this increase is associated with an increase in pulmonary wedge pressure[11]. Furthermore an observation on high level of GLE in the bronchoalveolar lavage fluid in ARDS has been reported[12]. Another report[13] documented that a negative correlation was noted between the changes in GLE and the postoperative cardiac index but positive correlation between the changes in GLE and A-a D_{O_2}. Therefore, it may be clinically beneficial to prevent the increase of GLE in the blood.

β-gl is another lysosomal enzyme, and its release into the blood indicates destruction of the lysosomal membrane due to tissue hypoxia. The principal role of the lysosomal enzymes is to digest metabolites produced within the cells or foreign bodies incorporated into the cells. However, the release of enzymes into the blood is supposed to induce a variety of pathophysiological conditions, namely impairment in circulation, respiration, vital-defensive function of the reticuloendothelial system[14,15].

Many membrane stabilizers and protease inhibitors have been used clinically for the treatment of such pathophysiological conditions. Corticosteroid is the most widely known as membrane stabilizer[16], while aprotinin preparations, gavexate mesylate and urinastatin are commonly used as protease inhibitor. These drugs may inhibit the induction of various pathological situations or may block a vicious circle.

Urinastatin is a glycoprotein of about 67000 in molecular weight extracted from human urine. Its inhibitory effect on various proteases such as trypsin, chymotrypsin and GLE[17], direct stabilization effect on the lysosomal membrane and antishock effect[18] have been well recognized. In addition, a number of reports show that urinastatin inhibits elevation of GLE in open heart surgery under extracorporeal circulation[19]. In the present study, the increase in GLE concentration could be suppressed after the rewarming by using double doses of urinastatin of 6,000 u·kg^{-1} as compared with that in the control. The necessity of repeated administration of urinastatin was due to a relatively short half-life, about 45 min, of this drug. As mentioned above, Ohnishi et al.[18] have reported that urinastatin can stabilize lysosomal membrane *in vivo* and *in vitro*. However, we could not observe any inhibitory effect of this drug on β-gl release from lysosomes after the circulatory arrest. Inhibitory effect of this drug on the release of lysosomal enzymes into the blood has not been consistently accepted among the investigators, as yet. Further studies will be needed to confirm the therapeutic value of this drug in the future. Matsunaga et al.[20] reported that urinastatin of 5,000 u·kg^{-1} was effective for inhibition of the release of lysosomal enzymes during extracorporeal circulation. On the other hand, Doi et al.[21] reported that no effect was observed under the similar circumstance.

It is interesting that the urine output increased significantly in Group U-II patients during the warming period after the recirculation. In canine models of hemorrhagic and endotoxin shock, urinastatin has been shown to increase the renal blood flow with a consequent increase in the urine volume[22] and also, to maintain the renal blood flow with a resulting increase in urine output during hypotensive anesthesia[23]. This suggests that urinastatin may have an effect in protecting the functions of urinary tubules and improving glomerular function[24]. It was suggested that peripheral circulation had been well maintained to keep the steady excretion of urine during the warming period.

Various humoral mediators could produce a vicious circle by affecting each other. Therefore necessity of combined administration of multiple mediator inhibitors, but not sole administration has been emphasized and its clinical significance, has been also documented[25–27]. Among such inhibitors, urinastatin as a protease inhibitor is expected to play an important role in this respect.

To conclude, intermittent administration of urinastatin during open heart surgery was shown to be effective to inhibit the increase in the plasma level of GLE at rewarming period after deep hypothermia.

(Received Mar. 11, 1991, accepted for publication Nov. 7, 1991)

References

1. Chiba K: Changes of Lysosomal Enzyme Activity during deep hypothermia. Masui (Jpn J Anesthesiol) 31:1190–1203, 1982
2. Wakusawa R, Shibata S, Okada K: Simple deep hypothermia for open heart surgery in infancy. Canad Anaesth Soc J 24:491–503, 1977
3. Neumann S, Gunzer G, Hennrich N, et al: "PMN-elastase assay": Enzyme immunoassay for human polymorphonuclear elastase complexed with α_1-protease inhibitor. J Clin Chem Clin Biochem 22:693–697, 1984
4. Kato K, Yoshida K, Tsukamoto H, et al: Synthesis of P-Nitrophenyl-D-Glucopyranosiduronic Acid and Its Utilization as a Substrate for the Assay of β-Glucuronidase Activity. Chem Pharmaceut Bull 8:239–242, 1960
5. Baggiolini M, Schnyder J, Bretz U:

Lysosomal enzymes and neutral proteinases as mediators of inflammation. Advances in inflammation Research, Vol. 1 Raven Press, New York, 263, 1979
6. Seitz R, Wolf M, Egbring R, et al: Neutrophil elastase, thrombin and plasmin in septic shock. Adv Exp Med Biol 240:481–484, 1988
7. Antonsen S, Brandslund I, Clemensen S, et al: Neutrophil lysosomal enzyme release and complement activation during cardiopulmonary bypass. Scand J Thorac Cardiovasc Surg 21:47–52, 1987
8. Riegal W, Spillner G, Schlosser V, et al: Plasma levels of main granulocyte components during cardiopulmonary bypass. J Thorac Cardiovasc Surg 95:1014–1019, 1985
9. Horl WH, Steinhauer HB, Schallmeyer P: Plasma levels of granulocyte elastase during hemodialysis: Effects of different dialyzer membranes. Kidney Int 28:791–796, 1985
10. Harg WM, Schollmeyer P, Horl WH: Granulocyte activation during haemodialysis in the absence of complement activation: inhibition by calcium channel blockers. J Clin Invest 18:380–385, 1972
11. Shimai S, Takano T, Ohya C, et al: Clinical implication of plasma PMN elastase (α_1-proteinase inhibitor-elastase compelx) in patients with early stage of scute myocardial infarction. J Jap Soc I Med 76:383–390, 1987
12. Idell S, Kucich U, Fein A, et al: Neutrophil elastase-releasing factors in Bronchoalvealar lavage from patient with adult respiratory distress syndrome. Am Rev Respir Dis 132:1098–1105, 1985
13. Doi H, Okutsu Y, Kitamura S, et al: Changes in serum activities of granulocyte elastase, pancreatic secretory trypsin inhibitor (PSTI) and β-glucuronidase in patients undergoing cardiopulmonary bypass. Masui (Jpn J Anesthesiol) 37:344–351, 1988
14. Glenn TM, Lefer AM, Beardsley AC, et al: Circulatory responses to splanchnic lysosomal hydrolases in the dog. Ann Surg 176:120–127, 1972
15. Bitensky L, Chayen J, Cunningham GJ: Behavior of lysosomes in hemorrhagic shock. Nature 199:493–494, 1963
16. Clermont HG, Williams JS, Adams JT: Steroid effect on the release of the lysosomal enzyme acid phosphatase in shock. Ann Surg 179:917–921, 1974
17. Muramatsu M, Mori S, Matsuzawa Y, et al: Purification and characterization of urinary trypsin in hibitor, UT 168, from normal humor urine, and its cleavage by human uropepsin. J Biochem 88:1317–1329, 1980
18. Ohnishi H, Suzuki K, Niho T, et al: Protective effects of urinary trypsin inhibitor in experimental shock. Jap J Pharmacol 39:137–144, 1985
19. Nabuchi A, Kumon K, Tanaka K, et al: The effect of Urinastatin on the release of granulocyte elastase at cardiopulmonay bypass. Jap Intensive Care Med 12:201–209, 1988
20. Matsunaga H, Matsumoto H, Yagyu K, et al: Effect of retreatment with urinastatin on circulatory dynamics and endocrinal functions during extracorporeal circulation. Jap J Artif Organs 16:530–533, 1987
21. Doi H, Andoh T, Okutzu Y: Effects of Urinastatin on the serum activities of granulocyte elastase, β-glucuronidase and pancreatic secretory tripsin inhibitor in patient having open cardiac surgery. Masui (Jpn J Anesthesiol) 37:975–980, 1988
22. Miyahara T: Effect of urinary trypsin inhibitor, MR-20 on Hemorrhagic shock in dogs. Masui (Jpn J Anesthesiol) 32:943–995, 1983
23. Tanifuji Y, Shima H, Mishima K, et al: The effect of Urinastatin on renal blood flow and urine volume in deliberate hypotension anesthesia. Masui (Jpn J Anesthesiol) 37:577–582, 1988
24. Sanaka T: New approach to the management of ischemic acute renal failure, protective effect of protease inhibitor on ischemic acute renal failure, Textbook for the group training course in coun termeasures against

renal failure. J Int Coup Ag 17:1, 1986
25. Almqvist PM, Ekstrom B, Kuenzing M, et al: Increased survival of endotoxin-injected dogs treated with methylpredonisolone, naloxone and ibuprofen. Circ Shock 14:129–136, 1984
26. Sielaff TD, Sugerman HJ, Tatum JL, et al: Successful treatment of adult respiratory distress syndrome by histamine and prostaglandin blockade in a procine pseudomonas model. Surgery 102:350–357, 1987
27. Sielaff TD, Sugerman HJ, Tatum JL, et al: Treatment of porcine pseudomonas ARDS with combination drug therapy. Trauma 27:1313–1322, 1987