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Effects of a protease inhibitor, ulinastatin, on coagulation and fibrinolysis in abdominal surgery

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Abstract
Purpose. Ulinastatin is well known to inhibit the activity of polymorphonuclear leukocyte elastase (PMNE). The PMNE concentration correlates with the activities of coagulation and fibrinolysis. The purpose of the present study was to investigate the effects of ulinastatin, a protease inhibitor, on coagulation and fibrinolysis in abdominal surgery.
Methods. Thirty patients, aged 40 to 70 years, with American Society of Anesthesiologists (ASA) physical status I or II, scheduled for major abdominal surgery, were enrolled. Anesthesia was induced with midazolam and thiopental, and was maintained with sevoflurane, nitrous oxide in oxygen, and an epidural block. An infusion of ulinastatin, 6000 units·kg^{-1} in 30 min, was started 1 h after the start of surgery in the ulinastatin group (15 patients). In the control group (15 patients), no protease inhibitors were infused. White blood cell count; platelet count; prothrombin time; activated partial thromboplastin time; and plasma concentrations of PMNE, antithrombin (AT), fibrin/fibrinogen degradation product (FDP), fibrinogen, plasminogen, plasmin-α_2 plasmin inhibitor complex (PIC), and thrombin-antithrombin complex (TAT) were measured before, at the end of, and 12 h after surgery.
Results. TAT, PIC, and FDP after surgery were significantly lower in the ulinastatin group than in the control group. AT was decreased in the control group but not in the ulinastatin group, with significant differences between the two groups.
Conclusion. Ulinastatin could inhibit coagulation and fibrinolysis in abdominal surgery.

Key words Polymorphonuclear leukocyte elastase · Protease inhibitor · Ulinastatin · Coagulation · Fibrinolysis

Introduction

Ulinastatin, an acid glycoprotein with a high molecular weight of 67000 Dalton, is contained in the fresh urine of healthy humans, and is well known to inhibit the activity of polymorphonuclear leukocyte elastase (PMNE) [1,2]. Ulinastatin also suppresses the production of tumor necrosis factor-α (TNF-α) [3] and interleukins 6 and 8 [4]. Therefore, it is expected that ulinastatin could decrease inflammatory reactions. Ulinastatin is also reported to attenuate reperfusion injury in liver [5] and heart [6]. The PMNE concentration correlates with the activities of coagulation and fibrinolysis [7]. From these studies, we hypothesized that ulinastatin could inhibit coagulation and fibrinolysis by inhibiting PMNE activity. The purpose of the present study was to investigate the effects of ulinastatin on coagulation and fibrinolysis in abdominal surgery.

Patients and methods

After obtaining institutional approval and informed consent from the patients, we enrolled 30 patients, aged 40 to 70 years, with American Society of Anesthesiologists (ASA) physical status I or II, and without liver, renal, respiratory, or cardiac complications who were scheduled to undergo major abdominal surgery. The patients were divided into two groups randomly by an envelope method.

As premedication, atropine 0.01 mg·kg^{-1} (maximum 0.5 mg) and midazolam 0.05 mg·kg^{-1} were administered intramuscularly 15 min before the patients entered the operating room. An epidural catheter was inserted into an appropriate interspinal space. Anesthesia was induced with midazolam 0.1 mg·kg^{-1} and thiopental 4 mg·kg^{-1}, and endotracheal intubation was facilitated with vecuronium 0.15 mg·kg^{-1}. Anesthesia was maintained with sevoflurane 0.5% to 1.5%, nitrous oxide 4 l·min^{-1} in oxygen 2 l·min^{-1}, and an epidural block using 1% lidocaine. Concentrated red blood cells (MAP-CRC; Japan Red Cross Society, Tokyo, Japan) were transfused when the hematocrit decreased to less than

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Table 1. Demographic data of the patients

	Ulinastatin group	Control group
Age (years)	58 ± 7	56 ± 9
Sex (male/female)	8/7	6/9
Body weight (kg)	57 ± 8	56 ± 6
Type of surgery (number of patients)		
Total gastrectomy	7	8
Pancreaticoduodenectomy	3	4
Esophagectomy	1	1
Rectal resection	2	1
Total resection of urinary bladder	2	1
Duration of surgery (min)	325 ± 169	305 ± 183
Blood loss volume (ml)	1546 ± 955	1463 ± 1031
Transfused blood volume (ml)	987 ± 598	813 ± 679

Values shown are means ± SD or numbers of patients

30%. An infusion of ulinastatin (Miraclid; Mochida Pharmaceutical, Tokyo, Japan), 6000 units·kg^{-1} in 30 min, was started 1 h after the start of surgery in the ulinastatin group (15 patients). In the control group (15 patients), no protease inhibitors or placebo were infused. After surgery, patients were administered epidural bolus injections of 0.25% bupivacaine, as required for pain management, in the intensive care units.

The white blood cell count (WBC); platelet count; prothrombin time (PT); activated partial thromboplastin time (aPTT); and plasma concentrations of PMNE, antithrombin (AT), fibrin/fibrinogen degradation product (FDP), fibrinogen, plasminogen, plasmin-α_2 plasmin inhibitor complex (PIC), and thrombin-antithrombin complex (TAT) were measured before, at the end of, and 12 h after surgery. Blood samples were drawn through an arterial catheter in the radial artery. The plasma concentration of PMNE was measured as the concentration of PMNE-α_1-antitrypsin complex, with an enzyme-linked immunosorbent assay (ELISA), using antibody for α_1-antitrypsin (Kitasato Biochemical Laboratory, Sagamihara, Kanagawa, Japan) [8]. AT was measured by a chromogenic peptide substrate method (COBAS; Roche, Tegimenta, Switzerland). FDP was measured by a latex agglutination method (COBAS FARA II; Roche). TAT was measured with an ELISA (ES-22; Boehringer Mannheim, Mannheim, Germany). PIC was measured with an enzyme immunoassay (UVIDEC-66; Nihon Bunko, Tokyo, Japan). Other parameters were measured at our central laboratory by routine methods.

Statistical analysis was performed with the χ^2 test for sex and type of surgery, one-way factorial analysis of variance (ANOVA) for other demographic data, and two-way repeated-measures ANOVA followed by the contrast as a post-hoc test for the measured variables. A P value of less than 0.05 was considered statistically significant.

Results

There were no differences in the backgrounds of the patients between the two groups (Table 1). No complication was observed after surgery in any of the patients.

No differences between the two groups were seen in any measured variables before surgery. WBC, PMNE, TAT, PIC, and FDP increased after surgery in both groups. TAT, PIC, and FDP after surgery were significantly lower in the ulinastatin group than in the control group. AT was decreased in the control group but not in the ulinastatin group, with significantly higher values in the ulinastatin group. In both groups, fibrinogen and plasminogen decreased after surgery, but there were no significant differences between the two groups. Platelet count, PT, and aPTT showed no significant changes during the study, and no differences between the two groups (Table 2).

Discussion

Ulinastatin inhibited the increase of TAT, FDP, and PIC, and it also inhibited the decrease of AT, while it had no effects on the other measured variables.

We administered ulinastatin 6000 units·kg^{-1} only once, because this dose is the maximum dose permitted to use in our country. In a clinical trial, 600 000 and 900 000 units were administered to humans without any side effects [9]. Therefore, 6000 units·kg^{-1} might be safely used.

TAT is produced from thrombin and has a rapid turnover; thus, it is a sensitive variable of the latent activation of the clotting pathways [10]. AT shows coagulation inhibitory capacity. Therefore, an increase in TAT and a decrease in AT means activation of coagulation. Ulinastatin could inhibit coagulation. FDP is derived from plasmin decomposition of fibrinogen and

Table 2. Parameters of coagulation and fibrinolysis and PMNE concentration

		Before surgery	At the end of surgery	12 h after surgery
WBC (mm^{-3})	Ulinastatin group	9812 ± 1501	16745 ± 1798*	14513 ± 1358*
(3700–9400)[a]	Control group	9657 ± 1262	15897 ± 1689*	13896 ± 1287*
PMNE ($\mu g \cdot l^{-1}$)	Ulinastatin group	115 ± 58	397 ± 187*	292 ± 135*
(21–165)[a]	Control group	122 ± 64	435 ± 212*	328 ± 174*
TAT ($\mu g \cdot l^{-1}$)	Ulinastatin group	10.1 ± 4.8	21.3 ± 8.7*;**	13.0 ± 5.7**
(<3.0)[a]	Control group	9.5 ± 6.7	43.5 ± 20.1*	23.8 ± 16.8*
FDP ($\mu g \cdot ml^{-1}$)	Ulinastatin group	8.4 ± 3.7	11.2 ± 2.4*;**	9.3 ± 3.5**
(<13)[a]	Control group	7.6 ± 4.3	14.9 ± 4.1*	12.3 ± 2.8*
PIC ($\mu g \cdot ml^{-1}$)	Ulinastatin group	1.1 ± 0.7	2.1 ± 0.7*;**	1.7 ± 0.5*;**
(0.8)[a]	Control group	1.3 ± 0.4	2.9 ± 0.7*	2.2 ± 0.8*
Fibrinogen ($g \cdot l^{-1}$)	Ulinastatin group	3.52 ± 0.89	1.99 ± 0.78*	2.31 ± 0.80*
(1.8–3.7)[a]	Control group	3.71 ± 0.76	2.06 ± 0.80*	2.40 ± 0.76*
Antithrombin (%)	Ulinastatin group	109 ± 9	98 ± 10**	94 ± 13**
(84–123)[a]	Control group	101 ± 10	68 ± 19*	73 ± 18*
Platelet ($\times 10^4\, mm^{-3}$)	Ulinastatin group	19 ± 6	17 ± 6	15 ± 5
(14–38)[a]	Control group	21 ± 7	19 ± 5	17 ± 6
Plasminogen (%)	Ulinastatin group	97 ± 9	69 ± 10*	51 ± 9*
(80–120)[a]	Control group	95 ± 8	62 ± 9*	55 ± 7*
PT (s)	Ulinastatin group	10.6 ± 0.8	10.4 ± 0.6	10.3 ± 0.5
(10–12)[a]	Control group	10.9 ± 0.7	10.6 ± 0.5	10.4 ± 0.5
aPTT (s)	Ulinastatin group	32.5 ± 3.6	33.1 ± 4.1	33.8 ± 4.5
(<45)[a]	Control group	32.7 ± 4.2	33.3 ± 4.6	33.7 ± 4.7

* $P < 0.05$ vs the value before surgery; ** $P < 0.05$ vs control group
WBC, white blood cell count; PMNE, polymorphonuclear leukocyte elastase; TAT, thrombin-antithrombin complex; FDP, fibrin/fibrinogen degradation product; PIC, plasmin-α2 plasmin inhibitor complex; PT, prothrombin time; aPTT, activated partial thromboplastin time
[a] Normal range; mean ± SD

fibrin; thus, an increase in FDP means activation of fibrinolysis. PIC is produced from plasmin immediately after fibrinolysis is activated, and its increase indicates fibrinolysis. However, fibrinogen and plasminogen were not different between our two groups. This suggests that ulinastatin could not inhibit the production of FDP and PIC. PT, aPTT, and platelet counts did not change during the study in either of the groups. These results show that major abdominal surgery did not induce clinically meaningful changes in coagulation and fibrinolysis. Therefore, the clinical effects of ulinastatin on coagulation and fibrinolysis could not be confirmed from the results of this study.

In an animal experiment, ulinastatin $5000\, U \cdot kg^{-1} \cdot 4\, h^{-1}$ inhibited the increase in FDP; decreases in fibrinogen and platelet count; and prolongation of PT and aPTT in disseminated intravascular coagulation (DIC) induced by endotoxin [11]. Considering these results and the present data, it is possible that ulinastatin may inhibit coagulation and fibrinolysis, improving PT and/or aPTT in septic or DIC patients. However, in septic or DIC patients, usually many other agents affecting inflammation, coagulation, and fibrinolysis are administered. Therefore, we thought it would be difficult to investigate the sole effects of ulinastatin in septic or DIC patients. In addition, patients with thrombotic events such as deep venous thrombosis, a good indication for an ulinastatin study, are rare in our country. Patients with major vascular surgery receive large amounts of blood transfusion, which could have some effects on the results of an ulinastatin study [2]. Thus, we selected patients having major abdominal surgery, in whom the changes in the measured parameters were not enough to mimic sepsis or DIC.

PMNE is released from granulocytes during surgery [12]. PMNE binds to α_1-antitrypsin immediately after release and is inactivated [13]. We measured the concentration of PMNE-α_1-antitrypsin complex, not free PMNE. Therefore, generally, the measured concentration was considered to show already inactivated PMNE. However, the complex itself is also reported to activate neutrophils to release PNME [14]. Therefore, measurement of the concentration of the complex is still worthwhile, in order to know the effects of PMNE.

PMNE is reported to inhibit the activity of or to degrade fibrin [15], fibrinogen [16], platelets [17], AT [18,19], and coagulation factors [8,20]. Therefore, inhibition of the activity of the PMNE by ulinastatin [1,2] would induce coagulation and fibrinolysis. In the present study, however, ulinastatin did not decrease the PMNE concentration, which is consistent with the results in a study with blood transfusion [21]. PMNE might not mediate the suppression of coagulation and fibrinolysis by ulinastatin. Ulinastatin inhibited the TNF-α production of activated human monocytes [3]. TNF-α plays an important role in the regulation of the

balance of coagulation and fibrinolysis [22,23]. We did not measure TNF-α. However, either the inhibition of TNF-α or direct effects might be the mechanism of the inhibitory effects of ulinastatin on the increases in TAT, FDP, and PIC, and the decrease in AT.

In conclusion, ulinastatin could inhibit the activation of coagulation and fibrinolysis in abdominal surgery.

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