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Serum Granulocytic Elastase and Superoxide Dismutase Activity after Administration of Protease-Inhibitor to Postoperative Patients

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Summary Postroperative changes in the plasma levels of granulocytic elastase and superoxide dismutase were examined clinically. Seven patients who had undergone cholecystectomy and eight patients who had undergone subtotal esophageal resection were considered. The protease inhibitor ulinastatin was administered to four of the latter patients. The levels of granulocytic elastase were elevated, and those of superoxide dismutase, decreased, in the non-medicated patients who had undergone esophageal resection ($p<0.05$). Furthermore, in the patients given the daily medication of 3×10^5 units of urinastatin for more than 3 days postoperatively, the elevation of granulocytic elastase levels and the decline of the superoxide dismutase levels were markedly suppressed ($p<0.05$). On the other hand, granulocytic elastase levels of the plasma to which ulinastatin had been added *in vitro* did not decrease. It is considered that the medicament of ulinastatin may be useful in the prevention of tissue injury caused by granulocytic elastase in cases where a disproportion of proteases and endogenous protease inhibitors exists.

Key Words: postoperative progress, granulocytic elastase, superoxide dismutase, ulinastatin, tissue injury

In recent years, preoperative and postoperative managements have progressed greatly and many new antibiotics have been developed. However, multiple organ failure after major abdominal surgery still remains a serious problem. Proteases and free radicals are gaining attention as two of the toxic mediators that cause multiple organ failure. They induce acceleration of capillary epithelial permeabil-

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ity and the failure of microcirculation [1], and granulocytes are a notable source of both toxic mediators [2].

A large amount of proteases pass into the blood sream from the lysosomes due to disruption by ischemia or a toxic factor such as endotoxin. Proteases activate many complement components and decompose or consume the endogenous inhibitors. Also, they cause acceleration of vascular permeability and irritate the coagulation system. Furthermore, they cause unnecessary accumulation of phagocytes including polymorphonuclear leukocytes, resulting in stagnation of leukocytes.

The emission of toxic oxygen radicals such as superoxide anion is advanced in surroundings, where dismutation is restricted. Disruption of the lysosomes advances further and a vicious circle occurs. Superoxide dismutase (SOD) scavenges the free radical specifically and prevents tissue injury caused by them [3].

Granulocytes store a large amount of proteases including elastase, which a serine protease in its azurophil granules. A granulocyte discharges the protease when irritated by phagocytes or when suffering any damage. The elastase decomposes the connective tissue protein, immunoglobulins, and other proteins which are components of the coagulation system, and therefore causes exacerbation of tissue injury [4]. It is possible to obtain information such as the extent of inflammation and effects of therapy by quantitative measurement of this enzyme [5].

Administration of the protease inhibitors has been attempted as therapy for tissue injury [6]. Ulinastatin is known to inhibit granulocytic elastase *in vitro* [7]. However, the clinical significance of giving protease inhibitor as medication to postoperative patients is not obvious. For this reason, the progress of postoperative plasma levels of granulocytic elastase and SOD and effect of ulinastatin treatment were examined in this study.

SUBJECTS AND METHODS

Seven patients who had undergone cholecystectomy and eight patients who had undergone subtotal esophageal resection were used as subjects of the study. The same surgical team performed all of the operations. The clinical stages of the tumor were 1 or 2.

Ulinastatin was administered for more than three days postoperatively to four of the patients who had undergone subtotal esophageal resection. The amount of medication of ulinastatin was 3×10^5 units per day.

All of the patients showed no signs of infection or other complications pre- or postoperatively.

Blood was collected early in the morning of the day before and 1, 3, and 6 days after the operation. The plasma granulocytic elastase levels were detected by the enzyme-linked immunosorbent assay [8]. SOD levels were measured by the nitrous acid method [9].

Changes in the plasma granulocytic elastase level after the addition of ulinastatin *in vitro* were also examined. The incubation time was 15 min, and the final concentration of ulinastatin was 100 U/ml.

Results are given as mean±SEM. Statistical evaluation was performed by Student' *t* test, and *p* values <0.05 were considered significant.

RESULTS

The plasma granulocytic elastase level of patients who had undergone cholecystectomy rose postoperatively (Fig. 1). However, even the highest level was below 2,000 μg/liter, and returned to the preoperative level 6 days after the operation.

In patients who had undergone subtotal esophageal resection without ulinastatin administration, the level elevated sharply to 4,100±920 μg/liter on the 1st postoperative day, and it rose further on the 3rd postoperative day to 5,200±700 μg/liter, the highest level overall. It then decreased slightly on the 6th day, but the value was still high, at 3,700±640 μg/liter. The difference of the levels between the patients who had undergone cholecystectomy and subtotal esophageal resection were statistically significant. In the medicated patients who had undergone subtotal esophageal resection, the value was 2,660±540 μg/liter on the first postoperative day, 4,800±620 μg/liter on the third day, and 2,800±560 μg/liter on the 6th day, but these levels were lower than those of the non-medicated patients.

The plasma level of total SOD of the patients who had undergone cholecystectomy was 24.8±10.6 U/ml on the day before the operation (Fig. 2). On the first post-operative day the level was 25.6±9.2 U/ml.

The level of the non-medicated patients who had undergone subtotal eso-

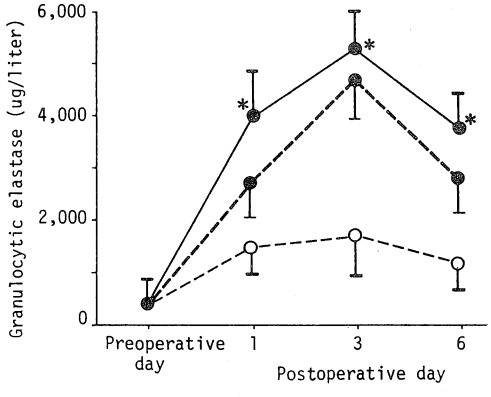

Fig. 1. Plasma granulocytic elastase levels after surgery. Mean±SEM, detected by enzyme-linked immunosorbent assay. o·····o, Patients who had undergone cholecystectomy, ($n=7$); ●——●, ulinastatin non-medicated patients who had undergone subtotal esophageal resection, ($n=4$); ●·····●, ulinastatin-medicated patients who had undergone subtotal esophageal resection, ($n=4$). $*p<0.05$.

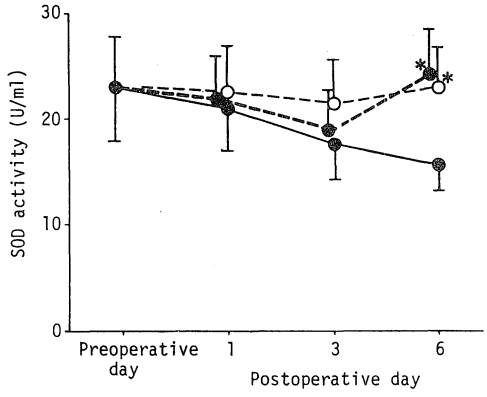

Fig. 2. Plasma superoxide dismutase levels after surgery. Mean±SEM, detected by the nitrous acid method. ○·····○, Patients who had undergone cholecystectomy, ($n=7$); ●——●, ulinastatin non-medicated patients who had undergone subtotal esophageal resection, ($n=4$); ●·····●, ulinastatin-medicated patients who had undergone subtotal esophageal resection, ($n=4$).*$p<0.05$.

phageal resection decreased on the first postoperative day. On the sixth day, the level was 15.4±2.6 U/ml, a significantly lower value than that of the patients who had undergone cholecystectomy. In the medicated patients who had undergone subtotal esophageal resection, the level decreased on the 3rd day. However, it recovered to 26.6±9.2 U/ml on the 6th day postoperatively, and this was significantly higher than that of the non-medicated patients. In addition, there was no significant difference between the levels of patients who had undergone cholecystectomy and those receiving subtotal esophageal resection with medication.

The granulocytic elastase levels of ulinastatin-treated plasma were not significantly lower than that of non-treated plasma (data not shown).

DISCUSSION

Postoperative plasma granulocytic elastase levels were elevated and the levels of SOD decreased in the non-medicated patients who had undergone subtotal esophageal resection. We conjecture that the main cause of this elevation is the acceleration of excessive rupture of leukocyte granules. The postoperative elevation of plasma granulocytic elastase levels and the decrease of plasma SOD levels were suppressed in the patients who received ulinastatin postoperatively, presumably due to a protection effect of ulinastatin on the granules. Of major significance is the finding that the decrease of SOD levels was suppressed by ulinastatin. These results suggest that this protease inhibitor may prevent the tissue injury.

Granulocytic elastase is usually complexed with an endogenous protease inhibitor in the plasma. In this condition, the elastase is not active. These inhibitors are α_1-antitrypsin (α_1-PI) and α_2-macroglobulin. We detected the im-

munoactivity of granulocytic elastase complexed with α_1-PI by the enzyme-linked immunosorbent assay. But we could not detect that of granulocytic elastase complexed with ulinastatin by the same method. If the binding between granulocytic elastase and ulinastatin is stronger than that of granulocytic elastase and α_1-PI, and we use an enzyme-linked immunosorbent assay that detects enzyme-α_1-PI complexes, the levels of granulocytic elastase in plasma treated with ulinastatin must show a decrease. However, after ulinastatin was added *in vitro*, the plasma granulocytic elastase levels did not show any decrease. Therefore, ulinastatin does not displace α_1-PI in this case. In the cases where endogenous protease inhibitors such as α_1-PI exist in sufficient amounts, treatment with an exogenous protease inhibitor such as ulinastatin would not prove to be significant. However, we conjecture that administration of an exogenous protease inhibitor such as ulinastatin will have a major effect in cases where a disproportion of proteases and endogenous protease inhibitors exists such as in the peripheral tissue.

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