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Modulatory Effect of a Serine Protease Inhibitor on Surgical Stress: Its Clinical Implications

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The relationship between endogenous cytokine antagonists and surgical stress is poorly understood. Surgical stress induces immunosuppression, and the reversed therapy of postoperative immunosuppression has been expected. The aim of the present study was to assess the effect of a serine protease inhibitor on postoperative immune reactivity. Twenty patients with colorectal cancer were randomly separated into experimental and control groups of 10 patients each. The experimental group received perioperative administration of a serine protease inhibitor while the control group did not. Plasma levels of cytokine antagonists, which suppress cell-mediated immunity, such as cortisol, interleukin-1 receptor antagonist, soluble interleukin-2 receptor (sIL-2R) and soluble tumor necrosis factors p55, p75 (sTNF-R55, -R75) were simultaneously measured. Significant reductions of plasma concentration of sIL-2R and sTNF-R55 were observed. Perioperative administration of a serine protease inhibitor may contribute to ameliorating immunosuppression after major surgery.

Key words: surgical stress, cytokine antagonist, protease inhibitor

Postoperative immune response is believed to be mediated by the proinflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF), and modulated by the naturally occurring specific antagonists of these cytokines (1). Postoperative production of TNF and IL-1 following major surgery has been studied by other researchers (2-5). In contrast, production of cytokine antagonists following surgical stress has not been extensively studied. Increased concentrations of soluble TNF receptor (sTNF-R), IL-1 receptor antagonist (IL-1Ra) and soluble IL-2 receptor (sIL-2R) have been reported in various pathological states, and are usually correlated with disease activity (6-8). Furthermore, recent reports have concluded that increased plasma concentrations of sTNF-R after major surgery are predictive of subsequent sepsis (9). The purpose of the present study was to investigate the serial changes of cytokine antagonists after surgery and the effect of perioperative administration of a serine protease inhibitor on postoperative production of cytokine antagonists.

Patients and Methods

Twenty patients were randomly separated into experimental and control groups of 10 patients each. The experimental group received a perioperative 24-h administration of intravenous serine protease inhibitor (FUT-175: 6-amidino-2-naphtyl p-guanidino-benzoate dimethanesulphonate; mol.wt. = 539.59, Torii Co. Ltd., Tokyo, Japan; protease inhibitor group). The control group received no protease inhibitor (control group). A serine protease inhibitor (FUT-175) was dissolved in 5% glucose solution and injected intravenously. Patients in the experimental group were started on an intravenous drip of a protease inhibitor at a dose of 0.1 mg/kg/h immediately before surgery for resection of colorectal cancer. Administration of a protease inhibitor was continued at the same rate during surgery and for 24 h after the completion of surgery. No patient was suffering from any another malignancy, autoimmune, neurological or connective tissue disease. Nor were any of them taking steroid or antihypertensive medication. All patients gave informed

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written consent. Table 1 outlines the patient characteristics. There were no significant intergroup differences.

Venous blood samples for serological assays were taken at 8:00 a.m. each morning. Samples were obtained on the day of surgery (day 0) and on postoperative days 1, 2 and 7. Plasma levels of IL-1Ra, sIL-2R and sTNF-R55, -R75 were quantified by commercial immunosorbent assay (ELISA) kits (IL-1Ra and sTNF-R55, -R75, Amersham Life Science; sIL-2R, T Cell Diagnostics, Inc.), and serum cortisol levels were simultaneously measured by radioimmunoassay using Gamma-Coat-Cortisol radioimmunoassay kits (Travenol Co. Ltd.).

Data are reported as the relative mean. Between groups, data were compared at defined time points using the Wilcoxon 2-sample test. A P value less than 0.05 was regarded as significant.

Results and Discussion

Previous studies have demonstrated a marked increase in plasma IL-6 post-operatively, but have been unable to detect a consistent rise in plasma IL-1β and TNF-α (10). Failure to detect IL-1β or TNF-α in plasma may be due to the transient, local paracrine release of these agents and their rapid proteolytic degradation (11). Alternatively, the assays may not be sufficiently sensitive, or may fail to detect receptor-bound or protein-bound cytokines (12). In severe disease and trauma, cytokines produced in excess or for prolonged periods may cause local and systemic toxicity (13). Their biological activity is normally controlled by regulation of biosynthesis and release, with the effects limited by possible counteractive mechanisms: autoantibodies, inhibitory or opposing cytokines, and soluble receptors or receptor antagonists.

In the present study, significant elevations in plasma levels of cytokine antagonists such as cortisol, sIL-2R, sTNF-R55, -75 and IL-1Ra on day 1 were observed after colorectal resection in the control group (Fig. 1). These cytokine antagonists would be expected to effectively neutralize their respective ligands in serum, based on their behavior *in vitro* (14-16). However, the capacity of cytokine antagonists to effectively neutralize their respective ligands in humans has not been well established (17, 18). As mentioned ealier, a recent report has concluded that increased plasma concentrations of sTNF-R55 after major surgery are predictive of subsequent sepsis (9). These results suggest that levels of postoperative cytokine antagonist production may be excessive and over the amount necessary to achieve full ligand antagonism under *in vivo* conditions. It has been reported that surgical stress induces immunosuppression, the mechanism of which might be, at least in part, due to the induction of these cytokine antagonists.

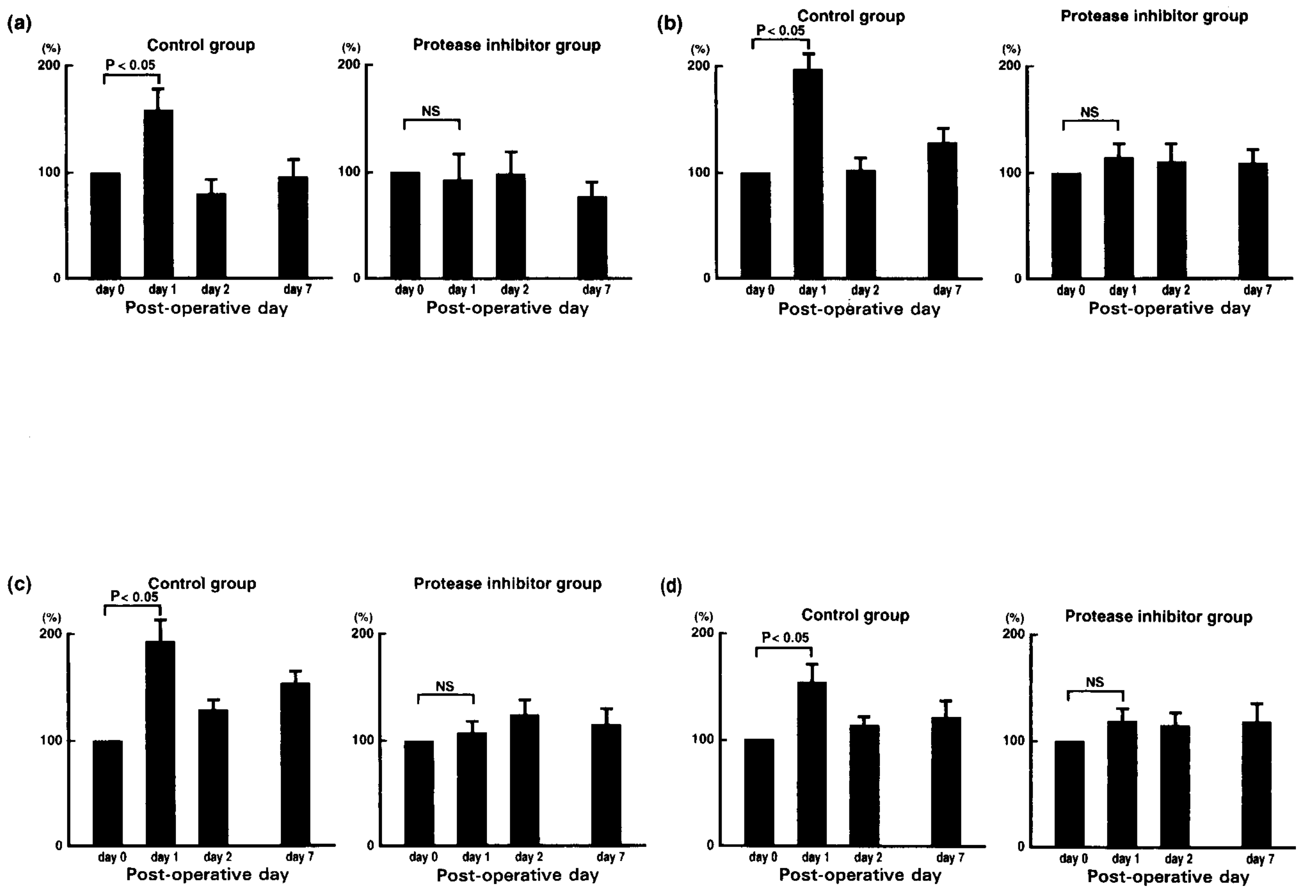
IL-2 is produced by mature T-lymphocytes in response to lectin or antigen activation (19-21) and promotes the in vitro growth of T-lymphocytes by interaction with IL-2-specific cell surface receptors (IL-2R) that appear in a time-dependent manner on activated T-cells (22). sIL-2R is released from activated human lymphoid cells *in vitro*, and markedly elevated levels of plasma sIL-2R have recently been reported in patients with hematologic malignancy (23-25) and autoimmunne disorders such as systemic lupus erythematosus (26). The plasma level of sIL-2R represents a good marker of disease activity and, apparently, the activity of a T-cell subpopulation (25, 26). sIL-2R binds with IL-2, thus making less IL-2 available for binding with IL-2 cell surface receptors (27).

sTNF-R is released by activated neutrophils (28), mononuclear blood cells and fibroblasts (29), by shedding from cell membranes. A recent report found that increased plasma sTNF-R55 concentrations one day after major surgery were predictive of subsequent sepsis and poor outcome, and that sTNF-R75 was less predictive (9,

Table 1 Profile of patients

	Protease inhibitor group	Control group	Significance
Sex (n; male/female)	2/3	6/9	NS
Age (years)	61.2 ± 4.9	54.6 ± 11.2	NS
Length of surgery (min)	257 ± 123.0	331.0 ± 136.3	NS
Blood loss (ml)	1319.0 ± 2078.5	1453.3 ± 1462.5	NS
Dukes (B/C/D)	1/2/2	4/5/6	NS

Values represent mean ± SD. Statistical significance was analyzed as appropriate.
NS: Not significant.



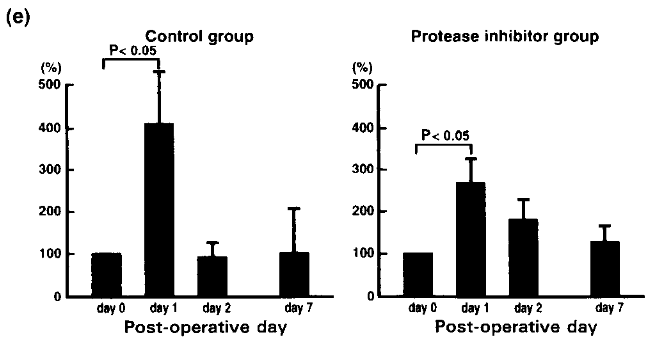


Fig. 1 The serial plasma levels of cortisol (**a**), soluble interleukin-2 receptor (**b**), soluble tumor necrosis factor receptor-type I (sTNF-R55) (**c**), soluble tumor necrosis factor receptor-type II (sTNF-R75) (**d**) and interleukin-1 receptor antagonist (**e**) following surgery with or without perioperative protease inhibitor treatment.
NS: significant.

30). Furthermore, plasma sTNF-R55 concentrations measured after liver transplantation reportedly increase before the development of severe infection (31). Plasma sTNF-R55 concentrations were significantly higher in the infected group than in the uninfected group after liver resection (32). Bacterial products such as formyl methionyl leucyl phenylalanine may be the primary inducers of sTNF-R release from neutrophils in patients with bacterial infection (28). Major surgery is reported to impair gut barrier function, resulting in increased bacterial translocation (32). Therefore, intraoperative bacterial translocation from the bowel may be a major stimulus causing the increased plasma concentration of sTNF-R.

Human monocytes produce IL-1β and IL-1Ra proteins in similar quantities (33). As monocytes mature to become macrophages, IL-1β production is downregulated and IL-1Ra production is greatly enhanced (34). Thus, tissue macrophages may be the major source of IL-1Ra after surgery. As described above, immune suppression has long been considered a consequence of major surgery (35, 36). Considering the key role played by IL-1 in influencing the growth and differentiation of immunocompetent lymphocytes, it seems reasonable to assume that the immunological suppression often observed after major surgery might be related to the IL-1Ra response which we have observed in the present study. A recent report of the ability of IL-1Ra to down-regulate IL-2, IL-2R expression and lectin-stimulated lymphocyte proliferation (37), would lend further support to the idea that IL-1Ra plays a role in modulating immune response.

IL-6 is a pleiotropic cytokine involved in the regulation of immune responses and the acute-phase reaction (38). This cytokine is produced by a variety of cells after stimulation, such as infection, trauma or immunological challenge. Previous reports have also identified IL-6 as the earliest detectable cytokine response associated with major surgery (32, 39). Earlier studies showed that IL-6 inhibits LPS-induced TNF-α and IL-1β production in cultured human monocytes, and in mice in vivo (40, 41), which suggest that IL-6 possesses antiinflammatory properties. A recent report also suggests that the antiinflammatory properties of IL-6 may be due to the induction of IL-1Ra synthesis and the release of sTNF-R (42). The same paper also suggests that tissue macrophages may be an important source of IL-6-induced cytokine antagonists.

In patients treated with perioperative administration of a protease inhibitor, with the exception of IL-1Ra, no significant increases of cytokine antagonists were observed (Fig. 1). The levels of IL-1Ra on day 1 in the protease inhibitor group were lower than those in the control group, although this was not statistically significant. The values of sIL-2R and sTNF-R55 in the protease inhibitor group on day 1 were significantly lower than those in the control group (Table 2). The low induction of soluble cytokine antagonists in the protease inhibitor group can be accounted for by two contrasting theories: The first holds that cytokine receptors are released from the cell surface through proteolytic cleavage, and therefore, that protease inhibitors may inhibit the shedding of cytokine receptors (43, 44). The second, described above, holds that IL-6 somehow causes the induction of cytokine antagonists such as IL-1Ra and sTNF-R (42). A serine protease inhibitor has been reported to cause a reduction in plasma concentration of IL-6,

Table 2 Comparison of plasma levels of cortisol, soluble interleukin-2 receptor (sIL-2R), soluble tumor necrosis factor receptor type I (sTNF-R55), soluble tumor necrosis factor receptor type II (sTNF-R75) and interleukin-1 receptor antagonis(IL-1Ra) between the evperimental group (protease inhibitor group) and the control group

		Protease inhibitor group	Control group	Significance
Cortisol	day 0	100.0	100.0	—
	1	91.6 ± 24.6	159.9 ± 20.8	NS
	2	98.9 ± 21.5	79.5 ± 18.0	NS
	7	78.8 ± 16.2	93.4 ± 19.3	NS
sIL-2R	day 0	100.0	100.0	—
	1	113.2 ± 12.1	179.8 ± 16.5	$P < 0.01$
	2	107.5 ± 18.6	105.5 ± 6.9	NS
	7	108.2 ± 15.2	126.0 ± 11.8	NS
sTNF-R55	day 0	100.0	100.0	—
	1	104.2 ± 10.9	194.5 ± 22.3	$P < 0.05$
	2	120.6 ± 16.5	125.9 ± 7.3	NS
	7	118.4 ± 14.1	153.9 ± 9.8	NS
sTNF-R75	day 0	100.0	100.0	—
	1	118.5 ± 11.8	152.8 ± 18.2	NS
	2	116.9 ± 13.6	114.4 ± 6.8	NS
	7	118.8 ± 18.6	120.7 ± 17.0	NS
IL-1Ra	day 0	100.0	100.0	—
	1	257.3 ± 64.0	406.8 ± 120.7	NS
	2	178.0 ± 44.8	93.4 ± 37.0	NS
	7	126.1 ± 38.6	102.9 ± 101.4	NS

Values represent mean ± SE. Wilcoxon 2-sample test was used to evaluate the statistical significance of intergroup differences.

which, in turn, may reduce the plasma levels of cytokine antagonists.

It has been reported that patients who suffer postoperative complications have higher plasma levels of cytokine antagonists than those who do not suffer complications (30) and that there is a marked increase in plasma cytokine antagonists in patients at risk for developing multiple organ failure (45). The present research demonstrates that perioperative administration of a serine protease inhibitor yields a reduction in the postoperative induction of cytokine antagonists related to immunological depression. These results suggest that a protease inhibitor has protective effects against surgical stress induced immunosuppression.

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