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Factor XIII Therapy of Anastomotic Leak, and Circulating Growth Factors

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Abstract

Wound healing is far more rapid in the gastrointestinal tract than in the skin. Once dehiscence of a surgical anastomosis in the gastrointestinal tract occurs, the high collagenase activity in the gastrointestinal tract may delay wound healing and promote the formation of a nonhealing fistula. Because factor XIII promotes cross-linking of fibrin during the early phase of wound healing, we investigated the effect of factor XIII concentrate on 16 anastomotic leaks and a nonhealing fistula. A 240-U dose of factor XIII concentrate (Fibrogammin P) was administrated intravenously for 5 days. Factor XIII activity and plasma levels of epidermal growth factor (EGF), transforming growth factor (TGF)-β, and interleukin-6 were measured before treatment and 1 day and 7 days after the end of treatment. Clinical outcomes were evaluated on the basis of the findings of contrast radiography, computed tomography, and drainage volume.

Improvement relevant to the therapy was observed in 15 cases (88.2%). Factor XIII activity increased to more than 70% of the normal value in 11 cases (64.7%) but remained at 40% to 70% of the normal value in 6 cases (35.3%). Plasma EGF and TGF-β levels increased in patients with improvement but were unchanged in patients without improvement. Our findings suggest that factor XIII significantly accelerates wound healing of anastomotic leaks and nonhealing fistulas by increasing circulating growth factors after systemic administration.
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Key words: factor XIII, transforming growth factor-β, epidermal growth factor, wound healing, anastomotic leak

Introduction

Factor XIII is a plasma transglutaminase that circulates as an inactive tetramer composed of two catalytic A-subunits and two accessory B-subunits (A2B2)[1,2]. Factor XIIIa, activated by thrombin and Ca^{2+}, plays various physiologic roles, including those in hemostasis and wound healing. Its main role is in fibrin cross-linking during the final steps of blood coagulation. The adhesive proteins that are involved in assembling extracellular matrix are the physiologic factor XIIIa.

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Table 1 Clinical findings of the patients

Case	Age	Sex	Diagnosis	Operation	Complication	Prognosis
1	60	M	esophageal cancer	esophagectomy	leakage	alive
2	68	M	gastric cancer	total gastrectomy	leakage	alive
3	62	M	gastric cancer	distal gastrectomy	leakage	alive
4	61	M	gastric cancer	distal gastrectomy	leakage	alive
5	68	M	gastric cancer	total gastrectomy	leakage	alive
6	62	M	gastric cancer	total gastrectomy	leakage	alive
7	66	M	remnant gastric cancer	ileocolic anastomosis	leakage	alive
8	52	M	jejunal perforation	jejunectomy	leakage	alive
9	51	M	colon cancer	colectomy	leakage	alive
10	71	M	rectal cancer	low anterior resection	leakage	alive
11	55	M	rectal cancer	low anterior resection	leakage	alive
12	66	M	rectal cancer	low anterior resection	leakage	alive
13	72	M	rectal cancer	low anterior resection	leakage	alive
14	56	M	pancreatic cancer	pancreatoduodenectomy	leakage	alive
15	48	M	pancreatitis	drainage	fistula	alive
16	61	M	esophageal cancer	esophagectomy	leakage	died
17	81	F	gastric cancer	distal gastrectomy	leakage	died

Table 2 Factor XIII activity

Patient	Days after operation		Factor XIII activity (%)			Effect
	appearance of complication	Factor XIII administration	before treatment	Day1	Day7	
1	8	20	<40	>70	>70	improvement
2	9	16	<40	40 ～ 70	40 ～ 70	improvement
3	11	18	<40	40 ～ 70	40 ～ 70	improvement
4	7	13	40 ～ 70	>70	>70	improvement
5	7	13	40 ～ 70	>70	>70	improvement
6	6	10	<40	>70	>70	improvement
7	6	47	<40	>70	>70	improvement
8	7	20	<40	40 ～ 70	40 ～ 70	improvement
9	29	36	<40	>70	>70	improvement
10	8	23	<40	>70	>70	improvement
11	6	57	40 ～ 70	>70	>70	improvement
12	7	21	<40	>70	>70	improvement
13	9	28	<40	40 ～ 70	40 ～ 70	improvement
14	5	22	<40	>70	>70	improvement
15	10	35	40 ～ 70	>70	>70	improvement
16	5	41	<40	40 ～ 70	40 ～ 70	no change
17	12	74	<40	40 ～ 70	40 ～ 70	no change

The gastrointestinal tract consists of an inner mucosal lining surrounded by a smooth muscle layer and a serosa. Systemic and local determinants affect the anastomotic healing after surgical resection of the gastrointestinal tract. Among many local factors, collagenase plays an important role in determining the integrity of surgical anastomoses in the gastrointestinal tract and in suture-holding capacity in the first few days of healing after surgery[3]. Unfortunately, complications of healing are not rare and can be characterized as insufficient healing, such as anastomotic leaks and fistulas. Decreased plasma factor XIII levels after surgery have been related to abnormalities in wound healing. Treatment of anastomotic leaks and fistulas with factor XIII concentrate is effective, and factor XIII activity

Schedule	Before Treatment	Treatment	After Treatment	
			Day 1	Day 7
Treatment *FP240U × 5 days*		●●●●●		
Assessment *Contrast radiography* *CT* *Drainage volume*	●			●
Examination *Growth Factors (EGF, TGF-β)* *IL-6* *Factor XIII*	●		●	●

Fig. 1 Schedule of treatment, examinations, and assessments

should be increased to more than 70% of the normal value[4].

In this study we assessed the effect of administering factor XIII concentrate to patients with low plasma factor XIII activity and anastomotic leaks or fistulas and measured circulating growth factors before and after treatment.

Patients and Methods

Patients

The subjects of the study were 17 patients (16 men and 1 woman) with anastomotic leaks or fistulas after gastrointestinal surgery, who had normal serum protein and albumin levels but low factor XIII activity after the postoperative acute inflammation resolved. In 16 patients anastomotic leaks developed after esophagectomy (n=2), total gastrectomy (n=3), distal gastrectomy (n=3), jejunostomy (n=1), ileocolic anastomosis (n=1), colectomy (n=1), low anterior resections (n=4), and pancreatoduodenectomy (n=1), and 1 patient a nonhealing fistula developed after necrosectomy for severe pancreatitis (**Table 1**).

Methods

A 24-m*l* (240 U) dose of factor XIII concentrate (Fibrogammin P, Hoechst Japan, Inc., Tokyo, Japan) was intravenously injected for 5 days after the postoperative acute inflammation resolved (**Table 2**). Factor XIII activity and the plasma levels of epidermal growth factor (EGF), transforming growth factor β1 (TGF-β), and interleukin-6 (IL-6), as a marker of inflammation, were measured before treatment and 1 day (24 hours) and 7 days after the end of treatment. Clinical outcomes were evaluated with contrast radiography, computed tomography scans, and measurements of drainage volume 7 days after the completion of treatment according to previously published criteria[4] (**Fig. 1**).

Nutritional status was assessed with a prognostic nutritional index (=10 × albumin [g/d*l*] + 0.005 × total lymphocyte count [/mm^3])[5] (Onodera index), and the duration of systemic inflammatory response syndrome (SIRS), which is well correlated with surgical stress, was also evaluated[5–7]. The patients were divided into an improved group and a no-change group, and the two groups were compared.

Assays

Factor XIII activity was measured with the latex method. Plasma levels of EGF and TGF-β were measured with radioimmunoassay (R&D Systems, Inc., Minneapolis, MN, USA), and levels of IL-6 were measured with enzyme-linked immunosorbent assay (TFB, Inc., Tokyo, Japan).

Statistical Analysis

Data are expressed as means ± SD. The data were statistically analyzed with the Wilcoxon single-rank test and Student's *t*-test by using StatView software (version 5.0, Cary, NC, USA). A 5% significance threshold was adopted.

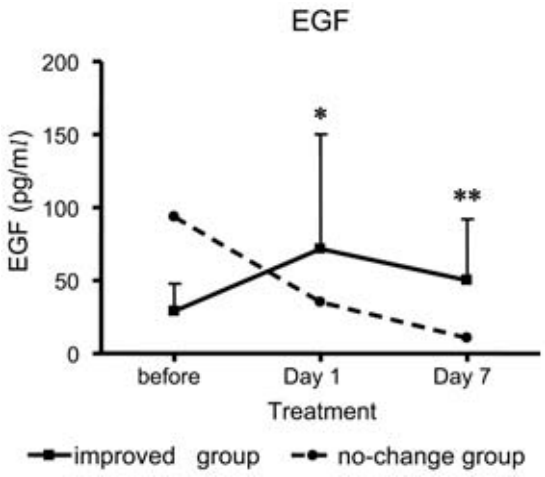

Fig. 2 Plasma EGF levels of patients with clinical improvement or no-change after factor XIII concentrate therapy.
*, vs. before treatment, p=0.03, **, vs. before treatment, p=0.04

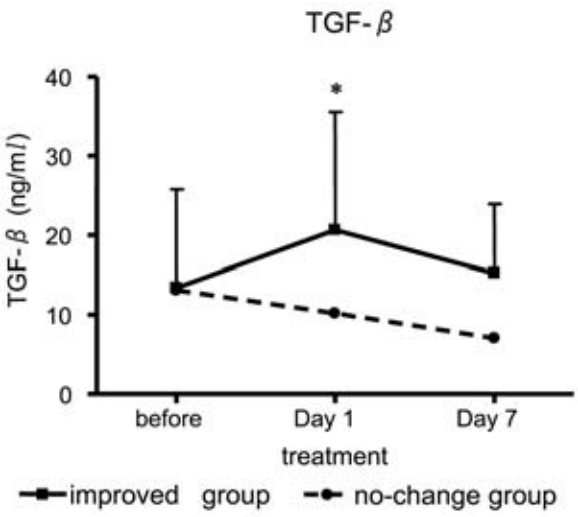

Fig. 3 Plasma TGF-β levels of patients with clinical improvement or no-change after factor XIII concentrate therapy.
*, vs. before treatment, p=0.03

Results

Clinical improvement of anastomotic leaks or a fistula was shown in 15 patients (88.2%) (improved group), and no change was shown in 2 patients (11.8%) (no-change group) (**Table 1**).

Nutritional Status and SIRS

There was no significant difference between the groups in serum total protein or albumin levels at any of the times measured. The prognostic nutritional index before surgery was 47.2 ± 9.6 in the improved group and 40.4 ± 8.2 in the no-change group (p=0.48). The duration of SIRS after surgery was 5.1 ± 3.2 days in the improved group and 8.5 ± 2.1 days in the no-change group (p=0.19).

Factor XIII Activity

After treatment factor XIII activity increased to more than 70% of the normal value in 11 cases (64.7%) but remained at 40% to 70% of the normal value in 6 cases (35.3%) (**Table 2**).

Plasma Growth Factors and Cytokines

In the improved group the plasma EGF level (pg/ml) was 28.9 ± 18.8 before treatment and significantly increased to 71.6 ± 78.4 (p=0.03) 1 day after the end of treatment and to 50.1 ± 42.0 (p=0.04) 7 days after the end of treatment (**Fig. 2**). The plasma TGF-β level (ng/ml) was 13.4 ± 12.4 before treatment and increased to 20.6 ± 14.9 (p=0.03) 1 day after the end of treatment and to 15.1 ± 8.7 (p=0.04) 7 days after the end of treatment (**Fig. 3**). There was no change in EGF or TGF-β after the end of treatment in the no-change group. The IL-6 levels were unchanged after the end of treatment in both groups (**Fig. 4**).

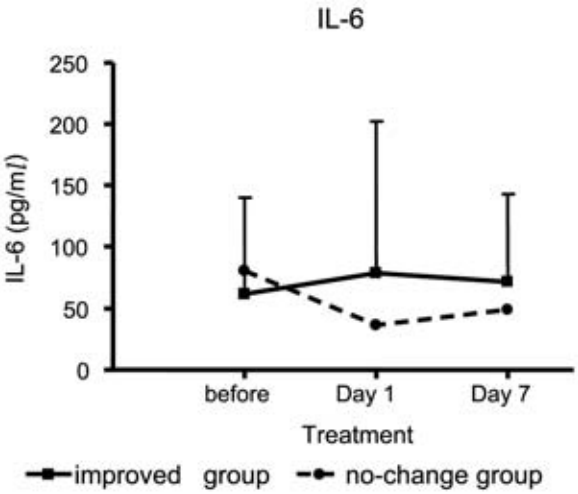

Fig. 4 Plasma IL-6 levels of patients with clinical improvement or no-change after factor XIII concentrate therapy.

Discussion

In this study patients with anastomotic leaks or fistulas had low plasma factor XIII activity after the inflammatory response. Administration of factor XIII increased plasma EGF and TGF-β levels and accelerated wound healing in the improved group, but there was no difference in the levels of these plasma growth factors or in wound healing after treatment with factor XIII concentrate in the no-change group.

The process of intestinal healing after surgical intervention is similar to that of skin healing and consists of: ① hemostasis and inflammation, ② proliferation, and ③ maturation or remodeling[8]. Numerous factors influence the healing of the gastrointestinal tract. The absence of serosa can contribute to a high incidence of anastomotic complications in the esophagus and rectum. Suture technique, such as serosa-to-serosa inverting or mucosa-to-mucosa everting, is also an important factor.

Factor XIII is a plasma transglutaminase that participates in the final step of the coagulation cascade. Thrombin-activated factor XIII is involved in numerous cross-linking reactions that result in fibrin network stabilization by cross-linking of the fibrin network[1,2]. In addition to its role in hemostasis, factor XIII is believed to participate in wound healing because of the defects in wound healing that occur in patients with inherited factor XIII deficiency[9,10]. Factor XIII is also important as a component of fibrin sealants, which improve fibrin cross-linking and clot strength[11]. Anastomotic leaks and nonhealing fistulas are believed to be stopped in the first hemostasis phase of wound healing .

A significant difference between the gastrointestinal tract and the skin is the role of intestinal smooth muscle cells, rather than fibroblasts, as collagen producers[12]. Another difference is that wound strength increases more rapidly in the gastrointestinal tract than in the skin[13]. Growth factors often have more than one effect on cells, such as chemotaxis of inflammatory cells and proliferation of fibroblasts in a concentration-dependent fashion. TGF-β is chemoattractive to monocytes in the femtomolar range, whereas the concentration necessary to increase collagen synthesis in fibroblasts lies in the nanomolar range[14]. TGF-β selectively upregulates collagen expression by intestinal smooth muscle cells but has no effect on their proliferation[15]. In addition, TGF-β inhibits epidermal cell proliferation but stimulates motility. EGF regulates the migration and growth of intestinal epithelial cells[16]. In this study treatment with factor XIII for 5 days increased levels of both TGF-β and EGF only 1 day after the end of treatment. The increase in growth factors is responsible for the proliferation phase of wound healing, because the factors are derived mainly from platelets and activated macrophages and are stored in the fibrin clot in normal wound healing. Once factor XIII activity increased to greater than 70% of the normal value, factor XIII consists in the stabilization of formed thrombi and in cell adhesion and migration and assembly of the extracellular matrix. Seven days after the end of treatment the leaks and a fistula showed improvement in the improved group, but there was no difference in plasma growth factors levels or clinical findings in the no-change group. Mishima et al have found that both factor XIII activity and its rate of increase before and after treatment are important[4]. These findings could contribute to the improvements in cases 2, 3, 8, and 13 despite low factor XIII activity, despite remaining unanswered in this study. Reasons for impaired wound healing might be far-advanced cancer and major leakage demonstrated with contrast radiography and drainage volume in cases 16 and 17. Systemic factors, such as age, malnutrition, atherosclerosis, circulatory impairment, and malignancy, rather than a local factor (factor XIII), likely played important roles in the delayed healing of the no-change group.

A reduction in intestinal wound marginal strength has been reported during the first 3 to 4 days after surgery[17]. This reduction is thought to be related primarily to increased collagenase activity in the wound site[18]. Venous leg ulcers are characterized by increased healing time and resistance to treatment. Topical treatment of venous leg ulcers with factor

XIII accelerates the healing rate and decreases fibrinolytic activity in wound biopsy specimen[19]. Thrombin-activated factor XIII promotes intestinal epithelial cell restitution through a TGF-β independent pathway in vitro[20]. Factor XIII concentrate has been found to increase the tensile strength of intestinal anastomoses and the number of *de novo* collagen fibers *in situ* on postoperative days 5 and 7 in an animal model[21]. Factor XIII concentrate may directly decrease fibrinolytic activity and enhance epithelial cell restitution and collagen synthesis.

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