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Short communication

Significance of chymase inhibition for prevention of adhesion formation

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Abstract

To clarify the role of chymase in adhesion formation, we investigated whether a chymase inhibitor could prevent adhesion formation after surgery in hamsters. Hamsters received a lesion produced by uterus scraping. A specific chymase inhibitor, 2-[4-(5-fluoro-3-methylbenzo[*b*]thiophen-2-yl)sulfonamido-3-methanesulfonylphenyl]oxazole-4-carboxylicacid (TY-51184), or placebo was injected into the abdomen before closing and scores for adhesion formation were assessed at 1, 4, and 12 weeks. A single peritoneal administration of TY-51184 significantly decreased the adhesion scores even at 12 weeks (placebo, 2.80 ± 0.20; chymase inhibitor, 1.60 ± 0.31). Thus, chymase inhibitors may be a novel strategy to prevent adhesion formation.

Keywords: Adhesion; Chymase; Chymase inhibitor; Surgery

1. Introduction

Postoperative intraperitoneal adhesions are a major cause of intestinal obstruction and infertility, and are thought to be one of the several serious complications of surgery. It is also a serious problem that the overall mortality rate among patients hospitalized with intestinal obstruction was reported to be 11.4%, and hospitalizations for adhesiolysis have been increasing (Dunn et al., 2001). Ray et al. (1998) reported that there were 303,836 hospitalizations in the United States in 1994 in which adhesiolysis was performed, accounting for 846,415 days of inpatient care and US$1.3 billion in hospitalization and surgeon expenditures. In a clinical analysis, half of adhesive obstructions occurred within the first postoperative month and the other occurred beyond the said period (Ellis, 1997). Therefore, prevention of adhesion formation is needed to reduce unnecessary morbidity and mortality rates, and it would be useful to identify or develop agents that prevent postoperative adhesions for a longer period.

Mast cells are inflammatory cells, and previous reports suggest that mast cells may be involved in peritoneal adhesion (Liebman et al., 1993). For example, the number of mast cells is increased around wounds in the late stages of the healing process; in contrast, mast cell stabilizers, which inhibit the activation and accumulation of mast cells, are effective in attenuating adhesion formation in rat models (Adachi et al., 1999). We also reported that adhesion formation in mast cell-deficient mice was significantly less severe than in normal mice (Yao et al., 2000). Recently, we reported that injection of Suc–Val–Pro–Phe^{P}(OPh)$_2$, which inhibits chymotrypsin-like serine proteases including chymase, into the abdomen only once during a surgical operation could prevent adhesion formation 2 weeks after the operation in a hamster adhesion model (Okamoto et al., 2002). However, it has been unclear how long a specific chymase inhibitor used only once during the operation will continue to suppress adhesion formation.

We determined whether a specific chymase inhibitor, 2-[4-(5-fluoro-3-methylbenzo[*b*]thiophen-2-yl)sulfonamido-3-methanesulfonylphenyl]oxazole-4-carboxylicacid (TY-51184), administered intraperitoneally, would prevent adhesion formation after surgery, and whether the preventive effect will continue for the long term.

2. Materials and methods

2.1. Agents and animals

A specific chymase inhibitor, TY-51184, was a kind gift of Toa Eiyo (Oomiya, Japan) (Koide et al., 2003). Mature

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female Syrian hamsters ($n = 60$; SLC, Shizuoka, Japan), 6 weeks of age and weighing 95–100 g, were maintained in an environmentally controlled room with a 12-h light/12-h dark cycle. The experimental procedure for the animals was in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College, Takatsuki City, Japan).

2.2. Surgical technique

Hamsters were anesthesized with intraperitoneal sodium pentobarbital (50 mg/kg). Surgical technique was performed using a modified procedure of Nagler et al. (1999). An abdominal midline incision was made, and bilateral uteri were grasped and denuded of serosa over the whole length of the bilateral uterine bodies until punctate hemorrhage occurred, using a swab.

To evaluate the preventive effect of a chymase inhibitor for the long term, in the chymase inhibitor-treated group, 1 ml of 100 μM TY-51184 in saline was injected into the abdomen, and then the abdomen was closed in two layers with silk thread. In the placebo-treated group, the same amount of saline as the chymase inhibitor was administered. Animals (placebo-treated group, $n = 10$; chymase inhibitor-treated group, $n = 10$, each term) were anesthesized with sodium pentobarbital (50 mg/kg, i.p.), and adhesions were assessed at 1, 4, and 12 weeks after the surgery.

2.3. Scoring of adhesions

The adhesions were graded by raters who were blinded to the treatment of any individual animal. Scoring was done according to a modified classification of Hulka et al. (1978): 0 = no adhesions; 1 = mild adhesions (filmy and relatively soft adhesions); 2 = localized moderate adhesions; 3 = moderate and wide adhesions; and 4 = severe adhesions that are impossible to separate.

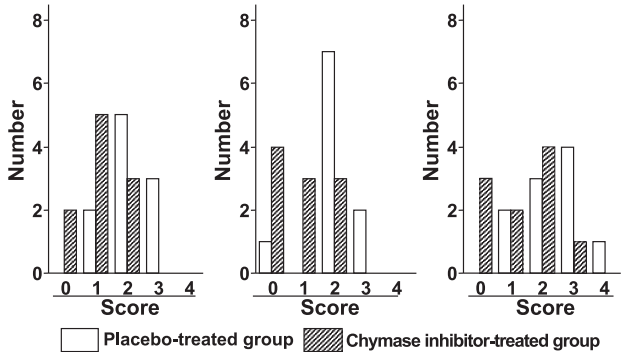

Fig. 1. The number of each adhesion score in the placebo-treated and chymase inhibitor (TY-51184)-treated groups at 1, 4, and 12 weeks after the operation. Data are expressed as the number of animals with adhesion score.

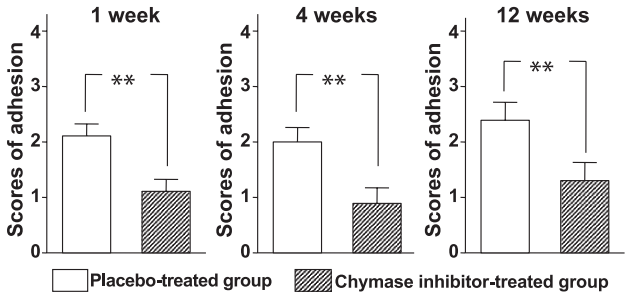

Fig. 2. The adhesion scores in the placebo-treated and chymase inhibitor (TY-51184)-treated groups at 1, 4, and 12 weeks after the operation. Values are mean ± S.E. $^{**}P < 0.01$ vs. placebo-treated group.

2.4. Statistical analysis

Adhesion scores were evaluated using the nonparametric Mann–Whitney U test. Values are given as mean ± standard error (S.E.). Differences were considered statistically significant at $P < 0.05$.

3. Results

As shown in Fig. 1, at all time periods after the operation, scores for adhesion formation between 2 and 3 were typically observed in placebo-treated hamsters. In chymase inhibitor-treated hamsters, scores typically observed were between 0 and 2.

The adhesion scores in the chymase inhibitor-treated hamsters were significantly lower than those in the placebo-treated hamsters at 1, 4, and 12 weeks after the operation (Fig. 2). In the placebo-treated group, the adhesion scores at 1, 4, and 12 weeks after the operation were 2.1 ± 0.23, 2.0 ± 0.26, and 2.4 ± 0.31, respectively, and were not significantly different. In the chymase inhibitor-treated group, the adhesion scores at 1, 4, and 12 weeks after the operation were 1.1 ± 0.23, 0.9 ± 0.28, and 1.3 ± 0.34, respectively, and were also not significantly different.

4. Discussion

Chymase is a chymotrypsin-like serine protease contained in the secretory granules of mast cells. A previous report demonstrated that chymase induces the accumulation of inflammatory cells such as neutrophils and eosinophils in vitro (He and Walls, 1998). After peritoneal tissues have been traumatized in a surgical procedure, accumulation of inflammatory cells is found within 12 h. New mesothelium begins to develop 2–3 days after the initial injury. The wound healing is normally completed within 7–9 days (Gomel et al., 1996). In the present study, the level of adhesion formation in the placebo-treated hamsters at 1 week was maintained even at 12

weeks after surgery. These findings suggest that postoperative adhesion formations are almost established in a week. Moreover, the antiadhesive effect of the chymase inhibitor at 1 week was also maintained at 12 weeks. Inhibition of chymase in the early phase after surgery may be important for the prevention of adhesion formation over the long term.

The chymase inhibitor used in this study, TY-51184, inhibits human, dog, and hamster chymases with IC_{50} concentrations of 37, 58, and 128 nM, respectively. However, this inhibitor does not inhibit chymostatin and cathepsin G even at 100 μM. Recently, we reported that a chymase inhibitor, Suc–Val–Pro–$Phe^{P}(OPh)_2$, prevented adhesion formation (Okamoto et al., 2002). However, this inhibitor inhibits chymotrypsin-like enzymes such as cathepsin G, which is contained mast cells and is not a specific inhibitor against chymase (Caughey, 1994). In this study, we demonstrated for the first time that a single treatment of a specific chymase inhibitor prevents adhesion formation.

In cultured human fibroblasts, the concentration of transforming growth factor (TGF)-β in the supernatant of media was significantly increased after injection of human chymase (Takai et al., 2003). Furthermore, human chymase dose-dependently increased cell proliferation, and this chymase-dependent proliferation was completely suppressed by a chymase inhibitor or an anti-TGF-β antibody (Takai et al., 2003). This report clearly demonstrated that chymase directly processes matrix-bound latent TGF-β to its active form and that this enzyme can induce the growth of fibroblasts. TGF-β may be related to adhesion formation. For example, TGF-β can induce intraperitoneal adhesion formation (Uchiide et al., 2002), and antibodies to TGF-β inhibit peritoneal adhesions in rat (Matsuzaki et al., 1998). The activation of TGF-β by chymase may contribute to the accumulation of extracellular matrix, resulting in the development and progression of adhesion formation. On the other hand, it is well known that the number of mast cells increases where inflammation occurs after surgery. We also reported that an accumulation of chymase-positive mast cells was observed in adhesion lesions, suggesting that the accumulation of chymase-positive mast cells may play an important role in the development of adhesion formation (Okamoto et al., 2002). Chymase activates stem cell factor, a typical cytokine that has the ability to induce the accumulation of mast cells. He and Walls (1998) reported that chymase also induces the accumulation of inflammatory cells such as neutrophils and eosinophils, both of which are related to tissue remodeling. Therefore, the inhibition of chymase by TY-51184 may suppress not only activation of TGF-β but also the accumulation of mast cells and other inflammatory cells, resulting in inhibition of adhesion formation.

In conclusion, a single intraperitoneal injection of a specific chymase inhibitor could prevent adhesion formation over the long term. Inhibition of chymase activity may become a novel strategy for prevention of adhesion formation.

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