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Short communication

Chymase inhibitor suppresses adhesion formation in a hamster experimental model

Yukiko Okamoto, Shinji Takai, Mizuo Miyazaki*

Department of Pharmacology, Osaka Medical College, Takatsuki City, Osaka 589-8686, Japan

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Abstract

To clarify the role of chymase produced by mast cells in adhesion formation, we investigated the preventive effect of a specific chymase inhibitor, Suc-Val-Pro-Phe^{P} $(OPh)_2$, on adhesion formation in a hamster experimental model. Hamsters underwent resection of the right uterine body and then 10 μM Suc-Val-Pro-Phe^{P} $(OPh)_2$ or placebo was injected into the abdomen. Two weeks after the operation, the scores for adhesion formation in the chymase inhibitor-treated group were significantly lower than that in the placebo-treated group (placebo-treated group, 3.60 ± 0.22; chymase inhibitor-treated group, 2.10 ± 0.22; $P < 0.01$). This specific chymase inhibitor, Suc-Val-Pro-Phe^{P} $(OPh)_2$, significantly suppressed the scores for adhesion formation in a hamster experimental model. Thus, chymase may play an important role in the adhesion formation.

Keywords: Adhesion; Chymase; Mast cell; Chymase inhibitor; (Hamster)

1. Introduction

Postoperative intraperitoneal adhesions are a well-known complication of surgery. Mast cells are known to be inflammatory cells and previous reports suggest that they may be involved in peritoneal adhesion formation (Persinger et al., 1983; Liebman et al., 1993). The number of mast cells is increased around wounds in the late stages of the healing process (Persinger et al., 1983; Liebman et al., 1993). 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). Recently, we also reported that adhesion formation in mast cell-deficient mice was significantly less severe than that in normal control mice (Yao et al., 2000). These reports suggest that mast cells are closely related to adhesion formation. However, mast cells release a large number of inflammatory mediators, such as histamine, serotonin, chemotactic factors, cytokines, and serine proteases, during the repair phase of adhesion formation (Ramos et al., 1991; Das et al., 1997). It has been unclear which factor might play an important role in the development of adhesion formation.

Chymase is a chymotrypsin-like serine protease contained in the secretory granules of mast cells. Previously, we reported that chymase activity was significantly increased in healing sites after cecal scraping in mice (Yao et al., 2000). However, the pathophysiological role of chymase in the development of adhesion formation remained unclear. In this study, to elucidate the relation between chymase and adhesion formation, we investigated the effect of a specific chymase inhibitor, Suc-Val-Pro-Phe^{P} $(OPh)_2$ (Oleksyszyn and Powers, 1991), on postoperative adhesion formation.

2. Materials and methods

2.1. Drugs and animals

The specific chymase inhibitor, Suc-Val-Pro-Phe^{P} $(OPh)_2$, was a gift from Dr. Oleksyszyn (Dyax Cambridge, MA, USA). Mature female Syrian hamsters ($n = 20$, SLC, Shizuoka, Japan), 6 weeks of age, weighing 85–90 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).

* Corresponding author. Tel.: +81-726-84-6418; fax: +81-726-84-6518.
E-mail address: pha010@art.osaka-med.ac.jp (M. Miyazaki).

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Table 1
Classification of adhesion formation

	Scores				
	0	1	2	3	4
Placebo-treated group	0	0	1	2	7
Chymase inhibitor-treated group	0	2	5	3	0

2.2. Surgical technique

Hamsters were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). An abdominal midline incision was made and the right uterine body was resected. In the chymase inhibitor-treated group, 1 ml of 10 μM Suc-Val-Pro-Phe^{P} $(OPh)_2$ in saline was injected into the abdomen. Then, the abdomen was closed in two layers with silk sutures. In the placebo group, 1 ml of saline was administered.

Two weeks after surgery, the animals (placebo group, $n=10$; chymase inhibitor group, $n=10$) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and then adhesions were assessed.

2.3. Scoring of adhesions

After anesthesia, the abdominal midline was incised and the intraperitoneal adhesion formation was scored. The scores for adhesion formation were graded blindly according to a modified classification of Hulka et al. (1978): score 0, no adhesions; score 1, mild adhesions; score 2, localized moderate adhesions; score 3, moderate and extensive adhesions; score 4, severe adhesions, impossible to separate.

2.4. Statistical analysis

Chymase activity was evaluated in a parametric test using Fisher's Protected Least Significant Difference. Adhesion scores were evaluated in a nonparametric test and statistically analyzed with the Mann–Whitney *U*-test. Values are given as means ± standard error (S.E.). Differences were considered statistically significant at $P<0.05$.

3. Results

In the placebo-treated hamsters, the scores for adhesion formation ranged between 2 and 4 and in particular, seven hamsters had a score of 4, which defines severe adhesions that are impossible to separate (Table 1). In the hamsters treated with the chymase inhibitor, scores of 4 were not found (Table 1). The scores for adhesion formation in hamsters treated with placebo and in hamsters treated with the chymase inhibitor were 3.60 ± 0.22 and 2.10 ± 0.23, respectively, and this difference was significant (Fig. 1).

4. Discussion

The chymase inhibitor, Suc-Val-Pro-Phe^{P} $(OPh)_2$, used in this study was characterized by Oleksyszyn and Powers (1991). The degradative half time of this inhibitor is about 20 h in human plasma (Oleksyszyn and Powers, 1994). Using dog vascular tissues, we reported that the IC_{50} value of Suc-Val-Pro-Phe^{P} $(OPh)_2$ for chymase was 2.8 nM (Takai et al., 2000). It is reported that chymase, an enzyme that is present in mast cell granules, is released immediately from the granules upon strong stimulation such as surgery, binds to extracellular matrix, and continues to function for several weeks (Craig and Schwartz, 1990; McEuen et al., 1995). The chymase inhibitor functions irreversibly, which means that the inhibitor, once bound to the enzyme, continues to inhibit it for a long time (Oleksyszyn and Powers, 1994). It is thought that chymase activity is fully inhibited for several weeks by treatment with this chymase inhibitor. In fact, we previously reported that chymase activity was significantly suppressed in vascular tissues for 4 weeks, when 10 μM Suc-Val-Pro-Phe^{P} $(OPh)_2$, which is the same dose as that used in the present study, was administered only during the operation (Takai et al., 2000). Therefore, in the present study, it is thought that the increased chymase activity in the injured uterus might have been inhibited for 2 weeks after the operation.

It is well known that the number of mast cells increases at sites where inflammation occurs after surgery. Previously, we reported that the accumulation of chymase-positive mast cells and the activation of chymase activity were observed in adhesion lesions (Yao et al., 2000). The accumulation of chymase-positive mast cells may play an important role in

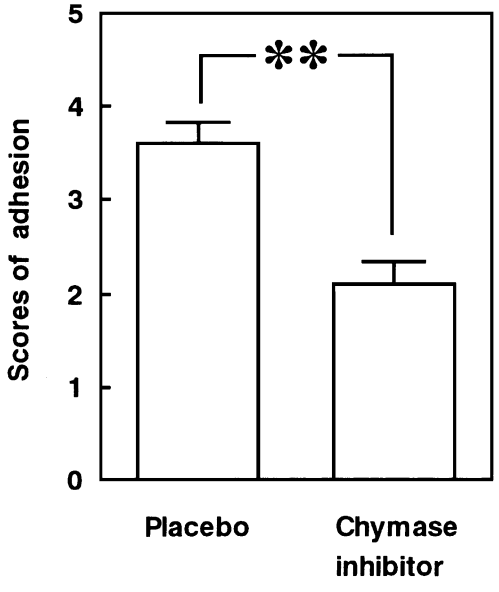

Fig. 1. The scores of adhesion formation in the placebo and the chymase inhibitor-treated hamsters. Values are means ± S.E. $**P<0.01$ vs. placebo-treated group.

the development of adhesion formation. 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 induces the accumulation of inflammatory cells such as neutrophils and eosinophils, both of which are known to be related to tissue remodeling. Therefore, in this study, the inhibition of chymase by Suc-Val-Pro-Phe^{p} $(OPh)_2$ may suppress the accumulation of mast cells and other inflammatory cells, resulting in the inhibition of adhesion formation.

The development and progression of adhesion formation are also known to cause tissue degradation and the growth of extracellular matrix. Chymase processes and activates promatrix metalloproteinase to form matrix metalloproteinase, which is a strong tissue degradative protease (Saarinen et al., 1994). Generally, however, matrix metalloproteinase is balanced by tissue inhibitor of metalloproteinase and tissue degradation is dependent on an imbalance between matrix metalloproteinase and tissue inhibitor of metalloproteinase. Chymase cleaves tissue inhibitor of metalloproteinase into inactive fragments and it also cleaves complexes of matrix metalloproteinase and tissue inhibitor of metalloproteinase, which have no matrix metalloproteinase activity, to form active matrix metalloproteinase (Frank et al., 2001). Activation of matrix metalloproteinase by chymase may contribute to the degradation of inflammatory lesions. Chymase directly cleaves type I procollagen to induce collagen–fibril formation and also processes matrix-bound latent transforming growth factor-β_1 to its active forms (Kofford et al., 1997; Taipale et al., 1995). Transforming growth factor-β_1 stimulates the expression of genes for collagen I, collagen III, and fibronection, all of which are related to the growth of extracellular matrix (Kim and Iwao, 2000). The collagen synthesis and the activation of transforming growth factor-β_1 induced by chymase may contribute to the accumulation of extracellular matrix, resulting in the development and progression of adhesion.

In conclusion, chymase plays an important role in the adhesion formation and the specific chymase inhibitor, Suc-Val-Pro-Phe^{p} $(OPh)_2$, may be a useful drug for suppression of peritoneal adhesions.

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