Oral Administration of a Novel Chymase Inhibitor, NK3201, Prevents Peritoneal Adhesion Formation in Hamsters

Yukiko Okamoto, Shinji Takai and Mizuo Miyazaki*
Department of Pharmacology, Osaka Medical College, Takatsuki City, Osaka 569-8686, Japan

Received April 5, 2002 Accepted July 6, 2002

ABSTRACT—We investigated the preventive effect of an orally active chymase inhibitor, NK3201 (2-(5-formylamino-6-oxo-2-phenyl-1,6-dihydropyrimidine-1-yl)-N-\{(3,4-dioxo-1-phenyl-7-(2-pyridyloxy))-2-heptyl\}acetamide), on the adhesion formation in a hamster experimental model. Hamsters were administered orally once daily with 30 mg/kg of NK3201 or placebo from 3 days before uterus scraping to 7 days after it. A significant increase of chymase activity in the injured uterus was reduced by treatment with NK3201. The score of adhesion formations in the chymase inhibitor-treated group was significantly decreased in comparison with that in the placebo-treated group (P<0.01). Oral administration of NK3201 may be a useful drug for prevention of peritoneal adhesion formation.

Keywords: Adhesion, Chymase, Mast cell

Chymase is a chymotrypsin-like protease contained in the secretory granules of mast cells. Chymase induces the accumulation of neutrophils, eosinophils and other inflammatory cells and directly cleaves type I procollagen to form collagen fibrils (1, 2). In postsurgical adhesions, the number of mast cells is increased around wounds in the late stages of the healing process, and it is thought to be involved in adhesion formation (3). 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, and it has been unclear which factor plays an important role in the development of adhesion formation. In this study, using an orally active chymase inhibitor, NK3201 (4), we investigated whether oral administration of the chymase inhibitor suppressed the adhesion formation.

NK3201 (2-(5-formylamino-6-oxo-2-phenyl-1,6-dihydropyrimidine-1-yl)-N-\{(3,4-dioxo-1-phenyl-7-(2-pyridyloxy))-2-heptyl\}acetamide) was a gift from Nippon Kayaku Co., Ltd. (Tokyo). Mature female Syrian hamsters (n = 51) (SLC, Shizuoka), 6 weeks of age and weighing 85 to 95 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).

Hamsters were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). An abdominal midline incision was made, and the right uterus was grasped and denuded of serosa over half the length of the uterine body until punctate hemorrhage occurred, using a swab (5). Then, the abdomen was closed in two layers with silk. Hamsters were administered orally once daily with placebo or 30 mg/kg of NK3201 from 3 days before uterus scraping to 7 days after it. Three days after the surgery, the animals (placebo group, n = 8; chymase group, n = 8) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and then the uterus was removed for the measurement of chymase activity. One week after the surgery, the animals (placebo group, n = 15; chymase group, n = 15) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and then adhesions were assessed.

A tissue extract for measurement of chymase activity was prepared as described previously (4). The uterus was minced and homogenized in 10 vol (w/v) of 20 mM Na-phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 30 min. The pellets were re-suspended and homogenized in 5 vol (w/v) of 10 mM Na-phosphate buffer (pH 7.4) containing 2 M KCl and 0.1% Nonidet P-40. The homogenate was centrifuged at 10,000 rpm for 30 min, and then the supernatant was used as a tissue extract.

Chymase activity was measured as described previously (6). A tissue extract was incubated for 30 min at 37°C with 4 mM angiotensin (Ang) I after preincubation in 150 mM
borax-borate buffer, pH 8.5, containing 5 mM ethylenediaminetetraacetic acid, 8 mM dipyridyl and 0.77 mM diisopropyl phosphorofluoridate, as inhibitors for angiotensin-converting enzyme and angiotensinases. The reaction was terminated with 15% trichloroacetic acid, and the mixture was centrifuged at 15,000 rpm for 5 min. The quantitation of His-Leu cleaved from an Ang I was determined using 10% o-phthaldialdehyde. One unit of chymase activity was defined as the amount of enzyme that cleaved 1 μmol His-Leu/min. Protein concentration was measured by the bicinchoninic acid protein assay reagent (Pierce Chemical, Rockford, IL, USA) using bovine serum albumin as a standard.

After anesthetization, the hamster was incised at the abdominal midline and the intraperitoneal adhesion formations were scored. The scores for adhesion formation were graded blindly according to a modified classification of Hulka et al. (7): Score 0, no adhesions; Score 1, mild adhesions; Score 2, localized moderate adhesions; Score 3, moderate and wide adhesions; Score 4, severe adhesions, impossible to separate.

Adhesion scores were evaluated in a nonparametric test and statistically analyzed by the Mann-Whitney’s U test. Values are given as means ± S.E.M. Differences were considered statistically significant at P<0.05.

The uterus chymase activity in the placebo-treated hamsters three days after the operation was significantly increased in comparison with that in the normal uterus. On the other hand, in the chymase inhibitor-treated hamsters, the chymase activity that had increased in the injured uterus was decreased, and we demonstrated that an oral administration of a specific chymase inhibitor significantly suppressed adhesion formation 1 week after the operation. Previous reports suggest that mast cells may be involved in peritoneal adhesion formation. For example, the number of mast cells is increased around wounds in the late stages of the healing process (3). A mast-cell stabilizer, which inhibit the activation and accumulation of mast cells, are effective in attenuating adhesion formation in rat models (8). These findings suggest that chymase contained in mast cells plays an important role in the development of the adhesion formation.

It is well known that the number of mast cells increases where inflammation occurs after surgery. An accumulation of chymase-positive mast cells and activation of chymase activity were observed in adhesion lesions (5). The accumulation of chymase-positive mast cells may play an important role in the development of adhesion formation. Chymase also induces the accumulation of inflammatory cells such as neutrophils and eosinophils, both of which are known to be related to tissue remodeling (9). Therefore,
the inhibition of chymase may suppress the accumulation of mast cells and other inflammatory cells, resulting in inhibition of adhesion formation.

The development and progression of adhesion formation are known to cause the growth of extracellular matrix. Chymase directly processes matrix-bound latent transforming growth factor (TGF)-β1 to its active forms (10). TGF-β1 stimulates gene expression of collagen I, collagen III and fibronectin, all of which are related to the growth of extracellular matrix (11). Furthermore, chymase directly cleaves type I procollagen to induce collagen-fibril formation (2). The collagen synthesis and activation of TGF-β1 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 development of adhesion formation, and an orally active chymase inhibitor, NK3201, may be a useful drug for suppression of peritoneal adhesions.

Acknowledgments

We thank Nippon Kayaku Co., Ltd. for the gift of NK3201. This study was supported in part by Grant-in-Aid 12770048 for Encouragement of Young Scientists of Japan.

REFERENCES

1 He S and Walls AF: Human mast cell chymase induces the accumulation of neutrophils, eosinophils and other inflammatory cells in vivo. Br J Pharmacol 125, 1491 – 1500 (1998)
2 Kofford MW, Schwartz LB, Schechter NM, Yager DR, Diegelmann RF and Graham MF: Cleavage of type I procollagen by human mast cell chymase initiates collagen fibril formation and generates a unique carboxyl-terminal propeptide. J Biol Chem 272, 7127 – 7131 (1997)
3 Persinger MA, Lepage P, Simard JP and Parker G: Mast cell numbers in incisional wounds in rat skin as a function of distance, time and treatment. Br J Dermatol 108, 179 – 187 (1983)
4 Takai S, Jin D, Nishimoto M, Yuda A, Sakaguchi M, Kamoshita K, Ishida K, Sukagen Y, Sasaki S and Miyazaki M: Oral administration of a specific chymase inhibitor, NK3201, inhibits vascular proliferation in grafted vein. Life Sci 69, 1725 – 1732 (2001)
5 Yao YL, Ishihara T, Takai S, Miyazaki M and Mita S: Association between the expression of mast cell chymase and intraperitoneal adhesion formation in mice. J Surg Res 92, 40 – 44 (2000)
6 Jin D, Takai S, Yamada M, Sakaguchi M, Yao Y and Miyazaki M: Possible roles of cardiac chymase after myocardial infarction in hamster hearts. Jpn J Pharmacol 86, 203 – 214 (2001)
7 Hulka JF, Omran K and Berger GS: Classification of adnexal adhesions: a proposal and evaluation of its prognostic value. Fertil Steril 30, 661 – 665 (1978)
8 Ramos BF, Zhang Y, Jakshik BA and Qureshi R: Mast cells are critical for the production of leukotrienes responsible for neutrophil recruitment in immune complex-induced peritonitis in mice. J Immunol 147, 1636 – 1641 (1991)
9 Adachi S, Maruyama T, Kondo T, Todoroki T and Fukao K: The prevention of postoperative intraperitoneal adhesions by tranilast: N-(3',4'-dimethoxy-cinnamoyl) anthranilic acid. Surg Today 29, 51 – 54 (1999)
10 Taipale J, Lohi J, Saarinen J, Kovanen PT and Keski-Oja J: Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. J Biol Chem 270, 4689 – 4696 (1995)
11 Kim S and Iwao H: Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. Pharmacol Rev 52, 11 – 34 (2000)