Development and Application of Chymase Inhibitors

Therapeutic Potential of a Specific Chymase Inhibitor in Atopic Dermatitis

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Abstract—A novel therapeutic mechanism may be the key to improving the chief symptoms and signs of atopic dermatitis (AD), which are persistent pruritus and high serum IgE. We demonstrate here that mast cell chymase may be a possible initiating factor and that the orally active specific inhibitor Y-40613 may have a therapeutic potential in the treatment of AD. We found that Y-40613 (2-[5-amino-2-(4-fluorophenyl)-1,6-dihydro-6-oxo-1-pyrimidinyl]-N-{1-[(5-methoxycarbonyl-2-benzoxazolyl)carbonyl]-2-phenylethyl}acetamide) dose-dependently suppressed the scratching response in a mouse pruritus model, with inhibitory efficacy enhanced by combination with conventional drugs, suggesting that chymase contributes to the development of pruritus by a unique mechanism or mechanisms. In fact, chymase injected in the model induced the scratching response. In vitro IgE production from mouse B cells was increased by purified rat chymase and suppressed by Y-40613. Increased serum IgE observed in Brown Norway rats injected with mercury chloride was suppressed by Y-40613. Furthermore, Y-40613 lowered ear thickness as well as serum IgE level in a mouse contact dermatitis model. Taken together, these findings suggest that the specific chymase inhibitor Y-40613 may ameliorate symptoms of AD through the dual inhibition of the chymase-dependent IgE production pathway and itching sensation.

Keywords: Chymase, Inhibitor, Atopic dermatitis

Mast cell chymase, synthesized and stored in mast cells located mainly in the heart, blood vessels, and skin, is a chymotrypsin-like serine protease. When activated by antigen-specific IgEs, mast cells secrete chymase from their storage granules along with the other protease tryptase and histamine (1). At the same time, they release platelet activating factor (PAF), leukotriene, and inflammatory cytokines and chemokines. These inflammatory factors injure local tissues directly or by inducing accumulation of eosinophils and lymphocytes. Although the pivotal role of mast cells in inflammation is well known, that of chymase remains unclarified. Suppression of chymase activity has therefore not been tested as a mechanism for anti-inflammatory drugs, which target mast cells.

Nevertheless, there have been recent reports on the role of chymase in the progression of inflammation, especially in chronic cases. These have observed that: 1) Chymase is stored in mast cells located in the skin; mast cell activation occurs in atopic dermatitis patients and the model animals (2); 2) When injected into human skin, chymase induces weal formation and pruritus (3); 3) When injected into guinea pig skin, it induces chronic vascular permeability which is resistant to antihistamines (4); 4) It degrades extracellular matrices directly and by activating matrix proteases (5, 6). More recently, the relation of BstXI polymorphism of chymase to atopic dermatitis has come under discussion (7–10).

We found that our specific chymase inhibitor, Y-40613 (2-[5-amino-2-(4-fluorophenyl)-1,6-dihydro-6-oxo-1-pyrimidinyl]-N-{1-[(5-methoxycarbonyl-2-benzoxazolyl)carbonyl]-2-phenylethyl}acetamide), suppress the production of IgE and pruritus, which are the two main symptoms of atopic dermatitis. Our investigations shown here sought to clarify whether specific chymase inhibitors have potential as a novel therapy for atopic dermatitis.
In vitro chymase inhibitory activity

We first determined the in vitro inhibitory profile of Y-40613 to chymase from human, dog, rat and mouse, and chymase-like proteases, chymotrypsin and cathepsin G. Enzyme activities were measured with respective synthetic substrates. Obtained Ki values are listed in Fig. 1. Y-40613 showed a broad spectrum toward chymases and chymotrypsin. On the other hand, Y-40613 at 10 μM did not inhibit human elastase, human thrombin, and human angiotensin converting enzyme.

Effects on mouse scratching behavior

We investigated the effects of Y-40613 on mouse scratching experiment, which is a model for pruritus (11, 12). BALB/c mice were sensitized by intravenous administration of anti-DNP IgE antibody (10 μg/mouse). Scratching behavior was induced by application of 0.75% 2,4-dinitro-1-fluorobenzene (DNFB) to the ears 24 h later. Behavior was monitored by a video camera for 30 min from 1 h after application of DNFB. The number of times the ear was scratched with the lower limb was counted. Test compounds were administered orally 1 h before application of DNFB. The portion above the dashed line represents IgE-dependent scratching behavior. Each value represents a mean ± S.E.M. (n = 7 or 8). *P < 0.05, **P < 0.01, vs control.

![Fig. 1. Inhibitory activity of Y-40613 on chymases and chymase-like proteases. In vitro inhibitory activities of Y-40613 on human, dog, rat and mouse chymase and the chymase-like proteases chymotrypsin (bovine) and cathepsin G (human) were assayed using the respective synthetic substrates. The rat and mouse chymases were extracted from peritoneal cells; the respective main components were expected to be RMCP-1 and MMCP-4.](image)

![Fig. 2. Suppression of mouse scratching behavior by Y-40613. Seven-week-old male BALB/c mice were sensitized by intravenous administration of anti-DNP IgE antibody (10 μg/mouse). Scratching behavior was induced by application of 0.75% 2,4-dinitro-1-fluorobenzene (DNFB) to the ears 24 h later. Behavior was monitored by a video camera for 30 min from 1 h after application of DNFB. The number of times the ear was scratched with the lower limb was counted. Test compounds were administered orally 1 h before application of DNFB. The portion above the dashed line represents IgE-dependent scratching behavior. At 30 mg/kg, Y-40613 suppressed this by approximately 80%. The anti-histamine ketotifen (1 mg/kg) showed only a non-significant degree of suppression. Suplatast (100 mg/kg), which is reported to have IgE suppressive activity, had no effect on scratching. Steroids and anti-histamines used as anti-pruritics also showed suppression of scratching. We investigated whether the chymase inhibitor Y-40613 shows enhanced suppression of scratching in combination with these drugs. The steroid prednisolone showed dose-dependent suppression of scratching. When Y-40613 (30 mg/kg) was used in combination with prednisolone, suppression was enhanced significantly (data not shown). Y-40613 also enhanced the scratching-suppressive effect of cyproheptadine serotonin /histamine antagonist.

It is reported that injection of chymase into human skin induces pruritus (3). We also observed that chymase injection induced scratching behavior in mice (data not shown). It is also reported that incubation of chymase with a skin specimen induces specific degradation of the structure between the dermis and the epidermis (13). Chymase may...
activate sensory nerves for pruritus directly or through production of some sort of activating peptides. Chymase inhibitors may prevent this process.

Effects on BN rat IgE production

Brown Norway (BN) rat is known to develop Th2 type reaction in response to injection of mercuric chloride (HgCl₂) accompanying production of serum IgE. Activation of mast cells is reported to be involved in this reaction (14, 15). We established a model, which produces approximately 100 μg/mL IgE, and examined the effect of chymase inhibitors on this production.

Y-40613 showed dose-dependent suppression of IgE production, which was statistically significant at doses of 10 mg/kg and above (Fig. 3). Suplatast (100 mg/kg) and ketotifen (1 mg/kg) showed a similar tendency, but the extent of suppression was small and not significant.

We have reported that rat mast cell protease-1 (RMCP-1), a rat chymase, enhances immunoglobulin E production by mouse B cells in vitro and the effect is suppressed in the presence of Y-40613 (16). This suggests that the target cell of chymase for IgE production might be B cells.

Effects on mouse DNFB-induced contact dermatitis

The effect of Y-40613 was measured in a mouse model of contact dermatitis, which served as a model of atopic dermatitis.

Contact hypersensitivity reactions were induced in male BALB/c mouse by applying 25 μL of 0.15% DNFB dissolved in acetone and olive oil to both surfaces of both ears once a week for 5 weeks. Compounds were orally administered concurrently for 29 days. Ear thickness was measured with a spring-loaded micrometer at 24 h after the fifth application of DNFB. Heel values represent a mean ± S.E.M. (n = 7 or 8). *P<0.05, **P<0.01, vs control.

Conclusion

Conventional drugs for atopic dermatitis are still insufficient in terms of efficacy or balance of efficacy and side effects. Steroids are the most effective drugs due to their strong anti-inflammatory activity. They are, however, well known to have the following potential side effects: contact dermatitis through sensitization of the skin, suppression of adrenal function in long-term treatment, and increased topical infection. Anti-histamines, meanwhile, which are usually used for the treatment of pruritus, may not show sufficient activity, especially in moderate or severe cases. Furthermore, certain formulations may induce sedative action or hepatic injury. Many pharmaceutical companies
have responded by attempting to develop modified compounds, while novel mechanisms of action have been explored to overcome the limitation of known mechanisms. In this report we showed that Y-40613, an orally active chymase inhibitor, suppresses the production of IgE and pruritus, which are the two main symptoms of the disease. Furthermore, our chymase inhibitor seems not to act on the H1 histamine receptor and therefore is unlikely to show sedative action, which is a major known side effect of antihistamines, and should be available for use in combination with anti-histamines to achieve stronger suppression of pruritus. Chymase inhibitors may have potential as a novel therapy for atopic dermatitis.

REFERENCES