Suppressive Effect of Tranilast on Interleukin-5 Prolonged Eosinophils Survival via Apoptosis

Gang Cheng, Takashi Ueda, Fukiko Eda, Syunichi Kinjyo, Hirokazu Nakajima, Yoshiki Ishii and Takeshi Fukuda

Department of Pulmonary Medicine and Clinical Immunology, Dokkyo University School of Medicine, Mibu-machi, Shimotsuga-gun, Tochigi 321-0293, Japan

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ABSTRACT—Tranilast has long been used clinically to treat allergic diseases such as bronchial asthma. To further clarify the antiinflammatory machanism, we examined the ability of tranilast to counteract the prolongation of eosinophil survival induced by interleukin (IL)-5. Tranilast reduced the IL-5 prolonged survival of eosinophils at the concentration range of 30 μg/ml to 100 μg/ml. The DNA extracted from eosinophils cultured with tranilast showed signs of fragmentation that was comparable with apoptosis. Electron-microscopic analysis of activated eosinophils cultured with 100 μg/ml of tranilast also revealed morphologic features of apoptosis. These data suggest that tranilast may act in vivo on activated eosinophils to reduce inflammation in allergic diseases.

Keywords: Tranilast, Eosinophil, Apoptosis

Tranilast has long been used clinically to treat allergic diseases such as bronchial asthma, atopic dermatitis and allergic rhinitis. The efficacy of tranilast in the treatment of these allergic diseases is based on the inhibition of antigen-induced chemical mediator release from mast cells and basophils (1 – 3). Recently, it has been reported that tranilast suppresses mitogen-induced activation of lymphocytes, interleukin (IL)-1 production from macrophages, IL-1-dependent fibroblast proliferation, IL-2 production from T cells and IL-2-dependent proliferation of lymphocytes (4). Miyachi et al. have also reported that tranilast inhibits the production of superoxide anion from human neutrophils (2). These findings suggested that tranilast inhibits not only the activation of mast cells but also those of monocytes, lymphocytes and leukocytes.

Eosinophils play important roles in allergic inflammation, including bronchial asthma (5). Eosinophilopoietic cytokines such as IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-5 are known to prolong the survival of eosinophils; and IL-5 has the most potent effects on eosinophil survival (6). To inhibit prolonged eosinophil survival might be useful in treating bronchial asthma. We previously reported that theophylline, glucocorticoids and macrolides suppressed the IL-5-induced prolongation of eosinophil survival by inducing apoptosis (7). Recently it was reported that tranilast inhibit PAF-induced eosinophil accumulation in pulmonary airways of guinea pigs (8). The bronchial hypersensitivity in asthmatics was suppressed by long term tranilast administration (9). These data suggested that tranilast may have some anti-inflammation effects in allergic disease.

However, the mechanism of anti-inflammatory effects of tranilast has not been clarified. We therefore studied whether tranilast inhibits the prolongation of eosinophils survival stimulated by IL-5. We also did an electron-microscopic study and an analysis of DNA fragmentation to determine whether the eosinophil apoptosis is induced by tranilast.

Eosinophils were isolated by the method described previously (10) with minor modifications, by using immunomagnetic beads (Miltenyi Biotec GmbH, Bergish Gladbach, Germany) and a magnetic cell separation system (MACS, Miltenyi Biotec GmbH) from peripheral blood of 4 asthma patients. We used anti CD-16 immunomagnetic beads as well as anti CD-3 immunomagnetic beads. After isolation, eosinophils were stained with trypan blue and counted in a hemocytometer. Cytospins of each preparation were also stained with Wright’s stain (Diff-Quick; International Reagent Corp, Kobe). Eosinophils were then resuspended in RPMI1640 medium with 10% heat inactivated fetal bovine serum, at a density of 1 × 10⁶/ml. The purity of the eosinophils was >98.8% ± 0.35%, and viability was >98%.
Recombinant human IL-5 was purchased from Pepro Tech EC Ltd. (London, England). The eosinophil survival assay was performed as reported previously (7). Tranilast was dissolved in a small amount of water containing 1% NaHCO₃ and added to RPMI medium (pH 7.4) to give a 100 µg/ml solution for this study. In brief, 200 µl of eosinophil suspension was put into the wells of a 96-well culture plate (Becton Dickinson, Lincoln Park, NJ, USA) with 100 pg/ml IL-5 and various concentrations of tranilast (0 – 100 µg/ml) and incubated in a humidified atmosphere at 37°C and 5% CO₂ for 96 h. To estimate eosinophil viability, the cells were double stained with fluorescein diacetate and propidium iodine and then counted with a hemocytometer and an epifluorescent microscope.

Cultured eosinophils (1 x 10⁶ cells) were fixed in 2.5% glutaraldehyde, further double fixed with 1% osmium tetroxide followed by dehydration, and routinely embedded. Thickly cut (1 µm) sections were stained with methylene blue, and the presence of the eosinophils was confirmed by light microscopy. The sections were further cut (500 nm) with a glass knife, were double-stained with uranyl acetate-lead citrate, and then were examined with an electron microscope (JEX100; Jeol, Tokyo).

DNA was extracted from eosinophils with an Apoptosis Ladder Detection kit (Wako, Osaka) according to the manufacturer’s instructions. The culture supernatants recovered from the tissue culture plates were assayed by means of the LDH-Cytotoxic test (Wako) according to the instructions of the vendors.

Data are expressed as the means ± S.E.M. Statistical analysis between groups was performed using the ANOVA test, and a P value <0.05 was considered significant.

The effects of tranilast on the survival ratio of eosinophils stimulated with IL-5 are shown in Fig. 1. Tranilast was found to inhibit IL-5 prolonged eosinophil survival in a dose-dependent manner (P<0.01). We also studied the effect of tranilast alone on eosinophil survival. Tranilast alone did not affect eosinophil survival significantly (data not shown). DNA fragmentation was observed to determine whether tranilast is capable of inducing eosinophil apoptosis. Eosinophils (1 x 10⁶ cells) were cultured for two days in the medium alone or in the presence of IL-5 (100 pg/ml) with or without tranilast (30, 100 µg/ml), and DNA was extracted. As shown in Fig. 2, no DNA fragmentation was observed in the sample cultured with IL-5 alone, but the DNA extracted from samples cultured with the combination of IL-5 and tranilast was definitely fragmented. To determine the decreased survival ratio of eosinophils incubated with tranilast that would result from apoptosis, we also observed the cultured eosinophils by electron microscopy. Most of the eosinophils, which had been cultured for 48 h with IL-5 (100 pg/ml) and tranilast (100 µg/ml), revealed microscopic changes consistent with condensation of nuclear chromatin and blebbing of the cell membrane, whereas these changes were rarely observed in eosinophils cultured with IL-5 alone (Fig. 3).

We demonstrated that tranilast inhibited IL-5-prolonged eosinophil survival and induced it via apoptosis. The dose of tranilast that has an inhibitory effect is 30 µg/ml, which can be reached clinically. These data led us to speculate that tranilast plays a role in the regulation of allergic inflamma-
It is well known that eosinophil-derived mediators are major contributors to the tissue damage underlying the inflammation responsible for much of asthma pathogenesis and the mechanisms involved in eosinophil accumulation are now considerable (11). Recently, much information about apoptosis has been obtained in the fields of allergy and immunology. Many kinds of cytokines have been identified, and among these, IL-5 is closely associated with eosinophils. Prolonged survival at sites into which eosinophils migrate is an important mechanism for activated eosinophils to exert a continuous effect.

We have also examined the cytotoxic effect of tranilast on eosinophils by means of the LDH cytotoxic test (data not shown), but the LDH cytotoxic test did not show altered levels of LDH in the samples cultured with 100 μg/ml tranilast compared with those cultured with medium alone. The characteristics of apoptosis consist of chromatin condensation in the nucleus and morphological changes such as aggregated chromatin, shrinkage of the cell, smooth cell surface and cytoplasmic vacuoles. At the same time, cellular DNA is destroyed by endonuclease, which cleaves the internucleosomal regions, forming a “ladder” of DNA. We have studied DNA fragmentation of eosinophils. Ladder formation was not observed in the cultured cells with IL-5 alone, but was detected in the cells cultured with tranilast, suggesting that the inhibition of tranilast on IL-5-induced eosinophil survival is due to apoptosis. Morphological study further confirmed the induction of apoptosis by tranilast in eosinophils activated with IL-5. This suppressive effect of tranilast on eosinophil survival is also consistent with the finding in vivo that tranilast inhibits PAF-induced eosinophil accumulation in airways of guinea pigs (8).

Although the mechanism by which tranilast causes apoptosis of IL-5-activated eosinophils is unknown, in vitro IL-5 can enhance eosinophil survival by abrogating apoptosis. IL-5 inhibits eosinophil apoptosis by the induction of new RNA and protein synthesis (6). Recent reports have indicated that Fas antigen can modify eosinophil survival by inducing apoptosis and its pathway is independent of the effect of IL-5 (12). Expression of Bcl-2 and its homologues in human eosinophils is modulated by IL-5 (13). Tranilast is known to increase the intracellular level of cAMP; and because d-cAMP induced eosinophil apoptosis after IL-5 stimulation (14), it is likely that tranilast induces apoptosis via elevation of intracellular cAMP in eosinophils. Recently, it was found that tranilast had an inhibitory effect on protein kinase C-dependent signal transduction in endothelial cells (15). It is considered to occur through a mechanism where tranilast affects IL-5 receptors through the inhibition of their signal transduction. Further study to clarify the precise mechanisms of tranilast-induced eosinophil apoptosis is needed.

In conclusion the present experiments revealed that tranilast has an inhibitory effect on IL-5-prolonged eosinophil survival and induces eosinophil apoptosis. This might be one of the anti-inflammatory effects of tranilast on active eosinophils that could be relevant in the treatment of allergic diseases.
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