Inhibitory Action of Tranilast, an Anti-Allergic Drug, on the Release of Cytokines and PGE$_2$ from Human Monocytes-Macrophages

Haruo Suzawa[1], Shinji Kikuchi[1], Kiyoshi Ichikawa[1] and Akihide Koda[2]

[1]*Pharmacological Laboratories, Kissei Pharmaceutical Co., Ltd., Hotaka, Nagano 399-83, Japan*
[2]*Department of Pharmacology, Gifu Pharmaceutical University, Gifu 502, Japan*

ABSTRACT — Tranilast, an anti-allergic drug that inhibits the release of substances such as histamine and prostaglandins from mast cells, has been reported to improve keloids and hypertrophic scars which originate from the abnormal proliferation and excessive collagen accumulation of fibroblasts. It has been considered that various chemical mediators produced by inflammatory cells play important roles in the development of keloids and hypertrophic scars. We therefore studied the effect of tranilast on the release of chemical mediators including transforming growth factor (TGF)-β_1, interleukin (IL)-1β and prostaglandin (PG) E$_2$ which are produced by the human monocytes-macrophages, and estimated whether these mediators induce collagen synthesis and cell proliferation of normal skin fibroblasts. Tranilast inhibited the release of TGF-β_1, IL-1β and PGE$_2$ from the human monocytes-macrophages. TGF-β_1 (25–200 pM) enhanced the collagen synthesis by fibroblasts. IL-1 (0.1–1 U/ml) increased the proliferation and conversely decreased the collagen synthesis. PGE$_2$ (2 μg/ml) enhanced the collagen synthesis. These results suggest that tranilast suppresses collagen synthesis by fibroblasts through inhibiting TGF-β_1 and PGE$_2$ production and cell proliferation by fibroblasts through inhibiting IL-1 production by inflammatory cells such as macrophages.

Keywords: Tranilast, Transforming growth factor (TGF)-β_1, Interleukin (IL)-1β, Macrophage, Collagen synthesis of fibroblast

Tranilast is well-known as an useful drug for improving and treating bronchial asthma, atopic dermatitis and allergic rhinitis. The efficacy of tranilast for the allergic diseases is based on the inhibition of chemical mediators release from mast cells and basophils during antigen-antibody reactions (1–8).

Several reports have shown that tranilast cures or prevents keloids and hypertrophic scars (9, 10). In experimental systems, tranilast decreases the weight of granulation tissues and inhibits collagen synthesis in human keloid tissues transplanted into the backs of nude mice and rat granulation-tissue induced by carrageenin (11–13). However, the precise mechanism regarding the inhibition by tranilast has not yet been clarified.

Keloids and hypertrophic scars are thought to be caused by abnormal proliferation and excessive collagen accumulation of fibroblasts (14–16). Many more mast cells are observed in hypertrophic scars and keloids compared to healthy human skin. Tranilast suppresses the histamine release from mast cells. For these reasons, much attention has been given to the roles of chemical mediators such as histamine and others from mast cells in the formation of keloids and hypertrophic scars (17, 18). Recently, however, it has been reported that mast cells are not required to enhance fibroblast growth because of the development of fibrosis in mast cell-deficient mice (19).

On the other hand, it has been considered that various cytokines produced by inflammatory cells including monocytes, macrophages and lymphocytes play important roles in the proliferation and collagen synthesis of connective tissue, and result in the formation of keloids and hypertrophic scars (20–22). There are few reports on the inhibitory effect of tranilast on cytokine production. Yanagi et al. (23) have reported that tranilast inhibits the production of interleukin (IL)-1 and IL-2 from human macrophages and T-cells.

In this study, we investigated the effect of tranilast on the production of cytokines by monocytes-macrophages from healthy volunteers, and estimated whether

cytokines, PGE_2 and histamine stimulate the collagen synthesis and proliferation of cultured fibroblasts derived from healthy skin.

MATERIALS AND METHODS

Drugs

Tranilast (N-(3,4-dimethoxycinnamoyl) anthranilic acid) was synthesized at the Kissei Pharmaceutical Company (Matsumoto). Histamine dihydrochloride, triamcinolone acetonide, lipopolysaccharide ($E.$ $coli$, 0127 : B8) and diphenhydramine hydrochloride were purchased from Wako Pure Chemicals (Osaka); transforming growth factor (TGF)-β_1 was from Kurabo Company (Osaka); IL-1β was from Kokusai Chemicals (Kobe); and prostaglandin (PG) E_2 was from Funakoshi Company (Tokyo). Tranilast was dissolved in 1% $NaHCO_3$ solution. Other drugs were dissolved in 99.8% ethanol, Dulbecco's modified Eagle's medium (DMEM) containing 0.1% bovine serum albumin (BSA) (0.1% BSA/DMEM) or DMEM containing 2% fetal bovine serum (FBS) (2% FBS/DMEM). All test drugs to be tested were diluted to the required concentrations and sterilized through a membrane filter.

Assay of TGF-β_1, IL-1β and PGE$_2$ released from monocytes-macrophages

Blood was taken from the ulnar veins of healthy male volunteers (27 – 40 years of age) and mixed with a 1/10 volume of 3.8% sodium citrate. A mono-poly resolving medium was used to collect mononuclear cells. The number of cells (10^6 cells/0.5 ml) was adjusted with calcium- and magnesium-free phosphate-buffered saline (PBS). According to the method of Yanagi et al. (23), the cell suspension was put into a 24-well tissue culture plate (Becton Dickinson Co., Ltd., Lincoln Park, NJ, U.S.A.). After incubation for 1 hr at 37°C under a 5% CO_2 atmosphere, the cell pellet on the plate was collected and used as monocytes-macrophages.

To induce the release of chemical mediators from the prepared monocytes-macrophages, the cells in the DMEM were cultured for 6 hr at 37°C in the presence of lipopolysaccharide (1 mg/ml) and the drug to be tested. Supernatant fluids were recovered by centrifugation. TGF-β_1, IL-1β and PGE$_2$ in the supernatant were measured by radioimmunoassay or enzyme-immunoassay.

Collagen synthesis of fibroblasts

Culture of fibroblasts: Healthy skin tissues obtained surgically from the vicinity of keloid tissue were aseptically sliced into fine pieces with ophthalmic scissors. Four or five slices were left in a 35-mm plastic plate

(Becton Dickinson). The primary culture of fibroblasts was performed at 37°C in 20% FBS/DMEM, penicillin (50 IU/ml), streptomycin (50 μg/ml) and kanamycin (100 μg/ml) under a humidified atmosphere of 5% CO_2 and 95% air. The culture medium was renewed every 3 – 4 days. Confluent cells were detached from the plates by the addition of 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA). Fibroblasts were routinely passaged every 5 to 7 days with 10% FBS/DMEM. The cultures at passage 6 to 8 (6 to 8th-passage cells) was used for the following experiments.

Collagen synthesis: Fibroblasts (4×10^5 cells) derived from healthy skin were disseminated into a 25-cm^2 tissue culture flask (Costar Co., Ltd., Cambridge, MA, U.S.A.) and cultured for 6 days in 10% FBS/DMEM. After they reached confluence, the cells were rinsed three times with PBS. To estimate collagen synthesis stimulated by TGF-β_1, IL-1β, PGE$_2$ and histamine, each reagent was added to the cultured fibroblasts derived from healthy skin. After the addition of l-ascorbic acid (50 μg/ml), the incubation was continued for 1 hr. The culture medium was replaced with 0.1% BSA/DMEM containing β-aminopropionitrile (100 μg/ml) and 37 kBq/ml of ^{3}H-proline (NEN), and fibroblasts in the medium were cultured for 6 hr further. Ten percent trichloroacetic acid, 0.5% tannic acid and FBS were added to the culture medium or to the cell homogenates, and the mixture was allowed to stand for 10 min. According to the bacterial collagenase digestion method of Peterkofsky and Diegelmann (24), the radioactivities of collagenase-sensitive protein (collagen) and the collagenase-insensitive (non-collagen) protein were measured. The relative synthetic activity of collagen was calculated by the following formula:

Relative synthetic activity of collagen (%) =

$$\frac{\text{collagen (dpm/}\mu\text{g cell protein)}}{\text{collagen (dpm/}\mu\text{g cell protein)} + 5.4 \times \text{non-collagen (dpm/}\mu\text{g cell protein)}} \times 100$$

The amount of protein in the cell homogenates was measured by the Bio-Rad protein assay.

Proliferation of fibroblasts: Fibroblasts (10^5 cells) after 6 – 8 passages derived from the healthy skin were suspended in 2% FBS/DMEM and disseminated in a 25-cm^2 culture flask (Costar). After 24 hr at 37°C, the medium was replaced with 2% FBS/DMEM containing TGF-β_1, IL-1β, PGE$_2$ or histamine at various concentrations, and cultivations were continued for 24 hr. The cells were removed with 0.25% trypsin-0.02% EDTA, and the number of cells was counted under a microscope after staining with 0.3% trypan blue. Cell viability was 94% or more.

Statistical analysis

The results obtained were expressed as the mean ± S.E. Student's t-test for paired observations was used to test for significance.

RESULTS

Effect on the release of TGF-β_1, IL-1β and PGE$_2$ from the human monocytes-macrophages

TGF-β_1, IL-1β and PGE$_2$ in the supernatant from cultured monocytes-macrophages stimulated by lipopolysaccharide (1 mg/ml) were measured. TGF-β_1, IL-1β and PGE$_2$ were released from monocytes-macrophages. Tranilast suppressed the release of TGF-β_1 at concentrations of 3–300 μM, PGE$_2$ at 30–300 μM and IL-1β at 300 μM (Figs. 1–3). Triamcinolone (1 μM) prevented the release of TGF-β_1 and exhibited a slight inhibition of IL-1β release. However, it had no effect on PGE$_2$ release (Figs. 1–3).

Collagen synthesis and cell proliferation caused by TGF-β_1, IL-1β, PGE$_2$ or histamine

Collagen synthesis: TGF-β_1 enhanced the collagen synthesis by fibroblasts derived from healthy skin at concentrations of 12.5–200 pM in a dose-dependent manner. PGE$_2$ (2 μg/ml) and histamine (0.1–1 mM) enhanced the collagen synthesis, but IL-1β (0.1 U/ml) inhibited the collagen synthesis (Fig. 4).

Cell proliferation: IL-1β at 0.1–1 U/ml enhanced the

proliferation of fibroblasts derived from healthy skin in a dose-dependent manner. Only TGF-β_1 at 50 pM increased the cell proliferation. On the other hand, PGE$_2$ and histamine had no effect on the proliferation (Fig. 4).
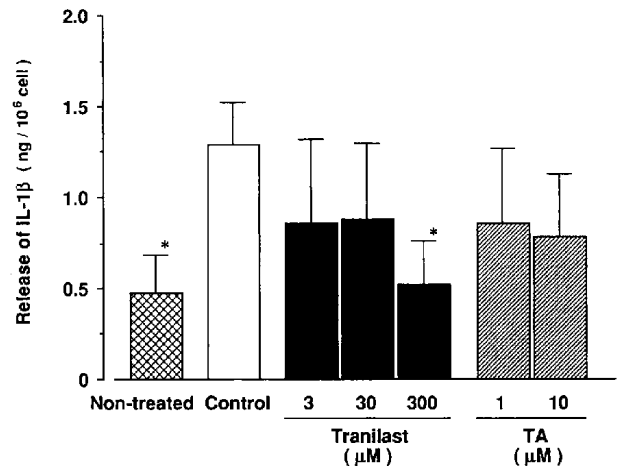

Fig. 2. Effects of tranilast and triamcinolone acetonide (TA) on the release of IL-1β from the human monocytes-macrophages by lipopolysaccharide (1 mg/ml). Each column indicates the mean ± S.E. for 6 experiments. *: significantly different from the control at P < 0.05.
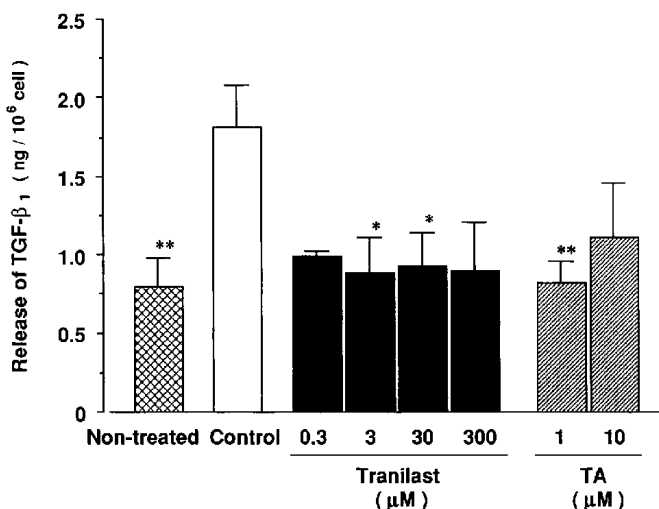

Fig. 1. Effects of tranilast and triamcinolone acetonide (TA) on the release of TGF-β_1 from the human monocytes-macrophages by lipopolysaccharide (1 mg/ml). Each column shows the mean ± S.E. for 3–7 experiments. * and **: significantly different from the control at P < 0.05 and P < 0.01, respectively.
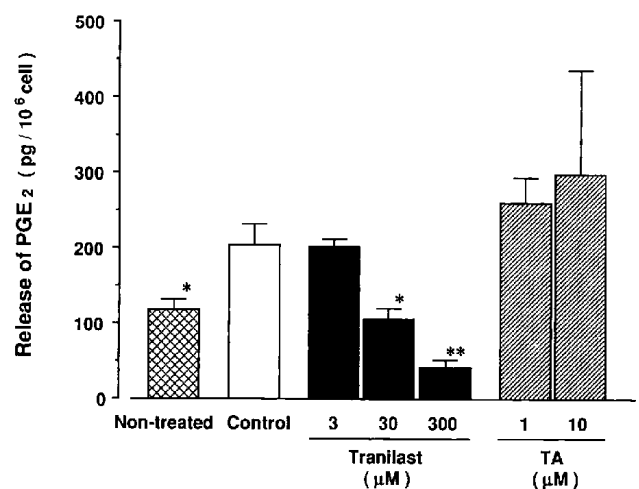

Fig. 3. Effects of tranilast and triamcinolone acetonide (TA) on the PGE$_2$ release from the human monocytes-macrophages by lipopolysaccharide (1 mg/ml). Each column indicates the mean ± S.E. for 4 experiments. * and **: significantly different from the control at P < 0.05 and P < 0.01, respectively.
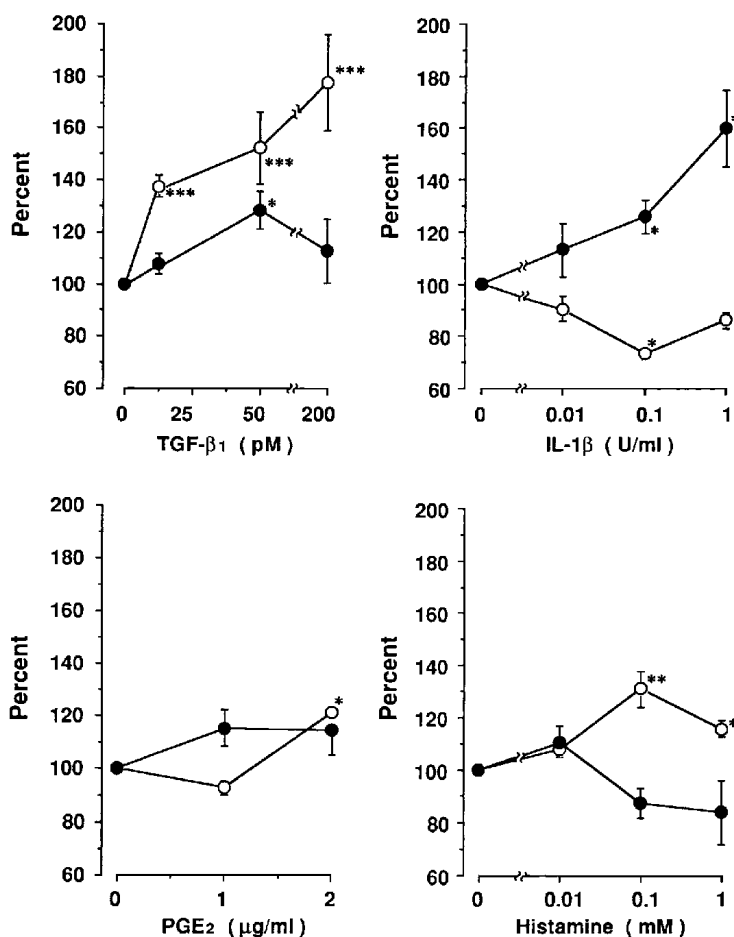
Fig. 4. Effects of TGF-β_1, IL-1β, PGE$_2$ and histamine on collagen synthesis and cell proliferation of the normal human skin fibroblasts in culture. Each point is expressed as the percentage of the value without stimulants and indicates the mean ± S.E. for 3–5 experiments. *, ** and ***: significantly different from the control (0) at P < 0.05, P < 0.01 and P < 0.001, respectively. ○: collagen synthesis, ●: cell proliferation.

DISCUSSION

It has been known that many kinds of inflammatory cell including mast cells, fibroblasts, lymphocytes, monocytes and macrophages are involved in chronic inflammation, and various cytokines produced by these cells play important roles in the acceleration or inhibition of chemotaxis, proliferation and collagen synthesis of fibroblasts (20–22). On the other hand, there are few reports on the inhibitory action of tranilast on the production of cytokines. Yanagi et al. (23) have reported that tranilast inhibits the production of IL-1 and IL-2, regulating cell proliferation from human macrophages and T-cells.

This study showed that TGF-β_1 and IL-1β are produced by the human monocytes-macrophages. IL-1β is produced mainly by monocytes and macrophages. In this study, IL-1β obviously enhanced the proliferation

of fibroblasts but only weakly inhibited the collagen synthesis. On the other hand, TGF-β_1 was first found in blood platelets, but it is also produced by monocytes and macrophages and has been recognized as a regulatory factor of cell proliferation (25, 26). This study as well demonstrated that TGF-β_1 is produced by monocytes-macrophages. Although TGF-β_1 enhances both fibroblast proliferation and collagen synthesis, the latter action is stronger (21, 22). In this study as well, with respect to human skin fibroblasts, TGF-β_1 showed a more pronounced effect in accelerating collagen synthesis than in enhancing cell proliferation. Keloids and hypertrophic scars result from abnormal proliferation of fibroblasts and excessive accumulation of collagen during the process of wound healing (11–13). If we consider this in connection with the results described above, the inhibition of collagen synthesis of fibroblasts by tranilast would involve the inhibitory ac-

tion on the release of TGF-β_1 produced by monocytes-macrophages. On the other hand, it would be likely that the inhibitory action of tranilast on the release of IL-1β results in suppression of fibroblast proliferation.

Tranilast inhibits the release of chemical mediators such as histamine, leukotrienes, prostaglandins and PAF, which are produced from mast cells and basophils during the antigen-antibody reaction. In this study, TGF-β_1, IL-1β and PGE$_2$ were released from the human monocytes-macrophages by lipopolysaccharide. TGF-β_1 and PGE$_2$ promoted collagen synthesis and IL-1β induced skin fibroblast proliferation. Tranilast at concentrations of $30-300\ \mu$M inhibited the release of TGF-β_1, IL-1β and PGE$_2$ from both cells. These concentrations ($30-300\ \mu$M) of tranilast are comparable to the concentrations ($10-1000\ \mu$M) that inhibit the release of histamine (5, 7).

Prostaglandins are important chemical mediators released from various inflammatory cells. Furthermore, attention has been focused on them as factors regulating fibroblast proliferation (27, 28). It has been reported that tranilast inhibits the production of prostaglandins by mast cells (8). PGE$_2$ was produced by human monocytes-macrophages and promoted the collagen synthesis by human skin fibroblasts. These findings suggest that in the incidence of hypertrophic scars, prostaglandins released from inflammatory cells such as macrophages, etc. are involved in the accumulation of collagen by fibroblasts.

Attention has been also focused on the involvement of histamine released from mast cells, because a large number of mast cells infiltrate into hypertrophic scars and keloid tissues and histamine enhances the growth of fibroblasts (17, 18). However, fibroblast proliferation is evoked in mast cell-deficient mice and anti-histaminergic drugs have no effects on the growth of fibroblasts (19). In this study, histamine had no effect on the cell proliferation, and collagen synthesis by fibroblasts was promoted at higher concentration of histamine (0.1 mM). Further studies are necessary to clarify the role of histamine in fibroblast proliferation and collagen synthesis in keloids and hypertrophic scars.

In conclusion, this study showed that tranilast inhibits the release from monocytes-macrophages of IL-1β, which enhances the proliferation of fibroblasts, and of TGF-β_1 and PGE$_2$, which enhance collagen synthesis. From these results, it would seem that tranilast inhibits the formation of keloids and hypertrophic scars through the suppression of collagen synthesis and cell proliferation of fibroblasts resulting from the inhibition of cytokine and prostaglandin release from macrophages and other inflammatory cells.

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