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SHORT COMMUNICATION

Suppressive Effects of Tranilast on the Expression of Inducible Cyclooxygenase (COX2) in Interleukin-1β-Stimulated Fibroblasts

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ABSTRACT. We investigated the effects of tranilast on inducible cyclooxygenase (COX2)-mediated prostaglandin E_2 (PGE_2) production and enzyme induction in interleukin-1β (IL-1β)-stimulated cultured dermal fibroblasts. IL-1β enhanced PGE_2 production in cultured fibroblasts. Tranilast did not affect constitutive cyclooxygenase (COX1) or COX2 activity in non-stimulated or IL-1β-stimulated fibroblasts. However, the COX2 expression induced by IL-1β was inhibited by tranilast. This result, that IL-1β-induced COX2 expression was suppressed by tranilast, was confirmed by immunohistochemical analysis. Thus, it is possible for tranilast to regulate PGE_2 production by inhibiting COX2 induction. BIOCHEM PHARMACOL 53;12:1941–1944, 1997.

KEY WORDS. inducible cyclooxygenase; prostaglandin E_2; tranilast; fibroblasts

Tranilast, an anti-allergic drug [1], has been reported to suppress the hyperproliferation of granulation tissue in keloids and hypertrophic scars [2]. Tranilast is thought to mediate these effects by inhibiting collagen synthesis and/or PGE_2§ production [3]. PGE_2 is also known to enhance collagen synthesis [4]. Therefore, tranilast may influence connective tissue (i.e. collagen) metabolism and inflammatory responses on keloid or hypertrophic scars by regulating arachidonate metabolism, but the mechanisms by which tranilast affects arachidonate metabolism (PGE_2 production) in human cultured normal dermal fibroblasts are not clear. It has been shown that two isozyme types (COX1 and COX2) exist in the PGE_2 production pathway [5]. COX1, which is referred to as the housekeeping enzyme, regulates mainly homeostasis, such as gastric acid secretion, whereas COX2 boosts the inflammatory response by COX2-mediated PGE_2. In the present study, we investigated the effects of tranilast on two types of cyclooxygenase-mediated PGE_2 production in cultured fibroblasts of cutaneous origin, according to the reports of Glaser and colleagues [6, 7]. In addition, we have discussed the effect of COX2-mediated PGE_2 on keloid and hypertrophic scar formation.

MATERIALS AND METHODS

Cultivation of Fibroblasts Derived from Human Skin

Normal skin aseptically obtained during surgery was cut into 2-mm squares and sterilized twice by immersion in PBS containing antibiotics (1000 U/mL penicillin, 1 mg/mL kanamycin, and 2.5 μg/mL amphotericin B). These sterilized fragments of skin were inoculated into culture dishes, covered with sterile cover glasses, and incubated in DMEM containing 10% fetal bovine serum at 37° in air containing 5% carbon dioxide. After confluence, cells were subcultured and the second passaged confluence fibroblasts were used for the experiments.

Influence of Tranilast on Cyclooxygenase Induction in IL-1β-Stimulated Fibroblasts

For inhibition of endogenous cyclooxygenase, confluent fibroblasts were incubated with serum-free DMEM containing 10 μM indomethacin for 15 min at 37° in air containing 5% carbon dioxide, and then were rinsed two times with PBS. Following the rinse, new DMEM containing 10% fetal bovine serum, 10 U/mL IL-1β, and 100 μM tranilast was added to the indomethacin-treated fibroblasts, and the cells were cultured for 5 hr at 37° in air containing 5% carbon dioxide. NS398 (2×10^{-7} M) and dexamethasone (4×10^{-8} M) were added to the cultured cells

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§ *Abbreviations:* PGE_2, prostaglandin E_2; COX1, constitutive cyclooxygenase; COX2, inducible cyclooxygenase; IL-1β, interleukin-β; DMEM, Dulbecco's modified Eagle's medium; and ABC, avidin-biotin-complex.

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instead of 100 μM tranilast as a positive control. After 5 hr of cultivation, indomethacin (final concentration 10 μM) was added to terminate the reaction. The media were promptly stored at −80° to assay for PGE_2 production.

Influence of Tranilast on Inducible Cyclooxygenase Activity in Fibroblasts

Confluent fibroblasts were incubated with serum-free DMEM containing 10 μM indomethacin for 15 min at 37° in air containing 5% carbon dioxide and rinsed two times with PBS, as previously described. The indomethacin-treated fibroblasts were cultured for COX2 induction with DMEM containing 10% fetal bovine serum and 10 U/mL IL-1β for 24 hr at 37° in air containing 5% carbon dioxide. After 24 hr, the medium was removed, and the IL-1β-stimulated fibroblasts were rinsed two times with PBS to remove residual IL-1β. DMEM (without serum) containing 10 μg/mL bradykinin as an arachidonic acid releaser and/or 100 μM tranilast was added to the rinsed fibroblasts, which were then incubated for stimulation of PGE_2 production at 37° in air containing 5% carbon dioxide for 60 min. NS398 and dexamethasone were also used as a positive control as described above. Specimens were stored as previously described.

PGE_2 Assay

Acetylcholinesterase-conjugated PGE_2 and PGE_2 monoclonal mouse IgGs were incubated with the specimen on micro plates precoated with goat anti-mouse polyclonal antibody, using a PGE_2 EIA Kit (Cayman Chemical Co., Ann Arbor, MI, U.S.A.) at ambient temperature for 18 hr. The plates were subsequently rinsed, and substrates for acetylcholinesterase were added to each well. After a 120-min incubation at ambient temperature, optimal density was determined with a micro plate reader (model 450, Japan Bio-Rad Co., Tokyo, Japan) at 410 nm. PGE_2 concentrations in the specimen were determined by absorbance, using calibration curves prepared simultaneously by an online data processor (Performa 575, Apple Co., Cupertino, CA, U.S.A.), and are expressed as picograms per well (mean ± SEM). The statistical significance of differences was examined by the unpaired *t*-test.

Immunohistochemistry

After cultured fibroblasts reached confluence, the cells were subcultured on cover glasses using the same methods. The cultured fibroblasts were treated as described under "PGE_2 Assay." The fibroblasts were rinsed three times with PBS and then fixed with 4% paraformaldehyde. The fixed fibroblasts were immunostained with polyclonal rabbit anti-murine COX2 antiserum (1:50 dilution, Oxford Biomedical Research Inc., Oxford, MI, U.S.A.). A Histfine SAB-PO (for rabbit) kit (ABC system, Nichirei Co., Tokyo, Japan) was used to localize the primary IgGs with oxidized diaminobenzidine as a chromogen, followed by a Mayer's hematoxylin counterstain.

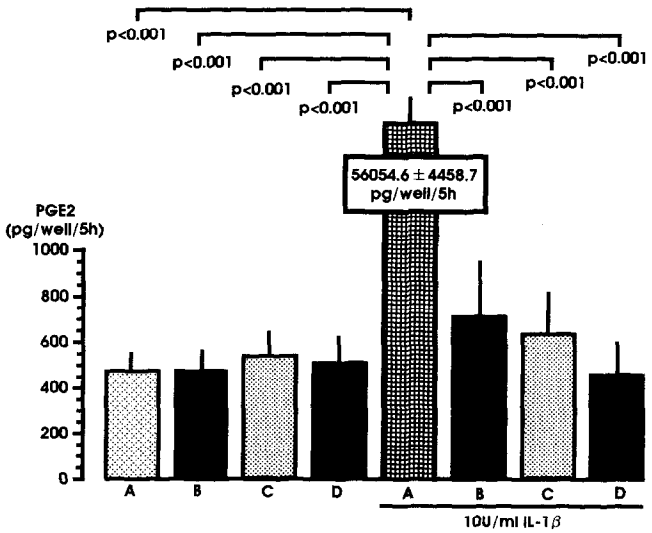

FIG. 1. Influence of tranilast on enzyme induction of cyclooxygenase 2 in IL-1β-stimulated normal human fibroblasts. Fibroblasts were cultured with IL-1β and/or the experimental drugs mentioned below for 5 hr at 37° under 5% carbon dioxide. The culture medium used was DMEM containing 10% fetal bovine serum. Each value is the mean ± SEM of four experiments. (A) Control (only DMEM), (B) tranilast (10^{-4} M), (C) dexamethasone (4×10^{-8} M), and (D) NS398 (2×10^{-7} M).

RESULTS

Influence of Tranilast on Cyclooxygenase Enzyme Induction in IL-1β-Stimulated Normal Human Fibroblasts

PGE_2 production in normal fibroblasts incubated with IL-1β for 5 hr was enhanced significantly compared with the control (Fig. 1). In contrast, PGE_2 production by IL-1β-induced type cyclooxygenase (i.e. COX2) was inhibited in fibroblasts cultured in medium containing IL-1β and tranilast under the same conditions for 5 hr. However, tranilast did not influence PGE_2 production in cultured fibroblasts under the no-IL-1β-stimulated condition. Although NS398 and dexamethasone, used as a positive control, also inhibited COX2-mediated PGE_2 production, they did not influence it under the no-stimulated condition.

Influence of Tranilast on Inducible Cyclooxygenase Activity of IL-1β-Stimulated Normal Human Fibroblasts

Tranilast did not affect PGE_2 production in fibroblasts stimulated by 10 μg/mL bradykinin, i.e. PGE_2 production of COX2 activity origin (Fig. 2). In conditions where the IL-1β-treated fibroblasts were stimulated with bradykinin, neither tranilast nor dexamethasone inhibited PGE_2 production by COX2 activity. However, NS398, a COX2 selective inhibitor, suppressed PGE_2 production in IL-1β-treated fibroblasts.

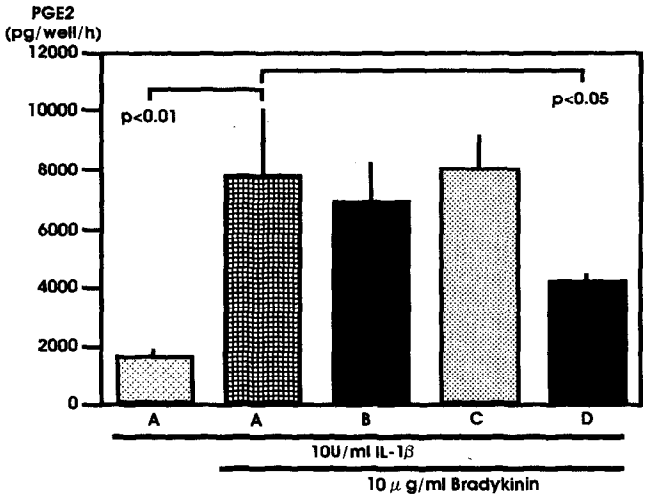

FIG. 2. Influence of tranilast on the enzyme activity of cyclooxygenase 2 induced by IL-1β. COX2 in fibroblasts was induced for 24 hr under the coexistence of IL-1β at 37°. To determine COX2 activity, fibroblasts in which the enzyme was induced by IL-1β were incubated with 10 μg/mL bradykinin (for the purpose of releasing the substrate, arachidonic acid) and experimental drugs (see B–D) at 37° for 1 hr. Each value is the mean ± SEM of four experiments. (A) Control (only DMEM), (B) tranilast (10^{-4} M), (C) dexamethasone (4×10^{-8} M), and (D) NS398 (2×10^{-7} M).

Immunohistochemistry

COX2-like immunoreactive proteins were detected in the cytosol of IL-1β-stimulated fibroblasts (Fig. 3, top panel). In contrast, COX2-like immunoreactive proteins were not detected in fibroblasts treated with IL-1β and tranilast (Fig. 3, bottom panel).

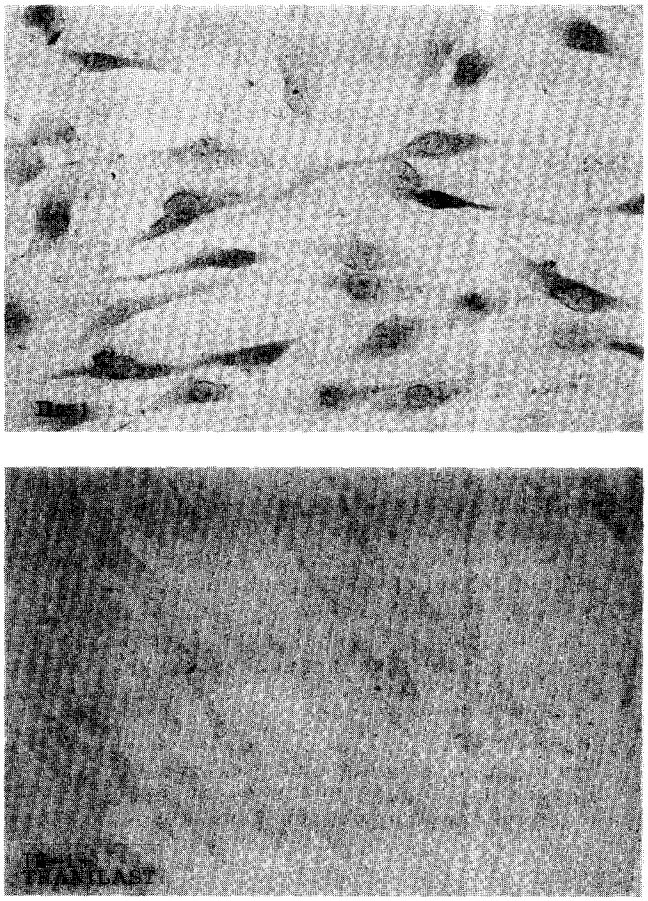

FIG. 3. COX2-like immunoreactivity in cultured fibroblasts. Top panel: fibroblasts treated with IL-1β. Bottom panel: fibroblasts treated with IL-1β and tranilast. Magnification: 400×.

DISCUSSION

Tranilast has been used clinically for the prophylaxis and treatment of cicatricial keloids and hypertrophic scars [2, 4]. This use has been justified, as antihistamines and corticosteroids are effective in improving symptoms such as erythema or prurius at the cicatrixation site. Moreover, mast cell infiltration, a known target of tranilast, has actually been observed at the affected site [8, 9].

Some of the chemical mediators released by mast cells promote the proliferation of fibroblasts and enhance metabolism in connective tissue [10]. Therefore, drugs that inhibit the release of chemical mediators can also inhibit the activation of fibroblasts by chemical mediators. IL-1β is known to activate fibroblasts, and it also has been detected in hypertrophic cicatricial tissue [11]. Thus, the effects of these factors on keloid formation are likely to be important. The present study was conducted to ascertain the effects of tranilast on inducible cyclooxygenase in fibroblasts stimulated by IL-1β. PGE_2 production is suppressed by phospholipase A_2 inhibitors, such as manoalide, as well as by cyclooxygenase inhibitors [12, 13]. The present results revealed that tranilast had no effect on basal PGE_2 production in fibroblasts (cultured in the absence of IL-1β). Although NS398, used as a positive control, significantly inhibited COX2-mediated PGE_2 production in fibroblasts stimulated by IL-1β for 24 hr, tranilast and dexamethasone had no effect. These results suggest that tranilast does not inhibit COX1 or COX2 activity. We also thought that tranilast could not inhibit phospholipase A_2 activity. However, tranilast added simultaneously with IL-1β significantly inhibited PGE_2 production in cells stimulated with IL-1β for 5 hr. These effects of tranilast coincided with the results showing that dexamethasone could not suppress COX2-mediated PGE_2 production but did inhibit the enzyme induction of COX2. It was also found, using immunohistochemical analysis, that tranilast could suppress the expression of COX2 protein by IL-1β. These findings suggest that tranilast, like dexamethasone, inhibits PGE_2 production by suppressing COX2 protein synthesis.

Dermal fibroblasts are also known to enhance collagen synthesis through cytokine-induced PGE_2. Therefore, the overproduction of COX2-mediated PGE_2 causes the stimulation of collagen synthesis, in the formation of keloids and hypertrophic scars. Thus, tranilast can suppress COX2 induction, probably at the transcriptional level, and may be important in COX2-mediated processes such as uterine maturation and keloid formation [14–16].

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