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European Journal of Pharmacology 498 (2004) 79–81

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Short communication

Inhibition by tranilast of nifedipine-induced proliferation of cultured human gingival fibroblasts

Toshimi Hattori*, Pao-Li Wang

Department of Dental Pharmacology, Matsumoto Dental University, Shiojiri 399-0781, Japan

Accepted 6 July 2004
Available online 20 August 2004

Abstract

The appropriate method of etiologic therapy for gingival overgrowth is yet unknown. In this study drug-induced proliferation of Gin-1 cells, a normal human gingival fibroblast cell line, was examined by using the reagent water-soluble tetrazolium-1. Tranilast (100 μM) inhibited the nifedipine (10 μM)-induced proliferation of gingival fibroblasts. The level of basic fibroblast growth factor (bFGF) was determined by using an enzyme-linked immunosorbent assay kit. Tranilast inhibited the release of bFGF from the cells. In conclusion, tranilast depresses the nifedipine-induced proliferation of gingival fibroblasts by inhibiting the release of bFGF. Administration of tranilast may thus be clinically effective for the treatment of gingival overgrowth.
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Keywords: Tranilast; Gingival fibroblast; bFGF; Proliferation

1. Introduction

Various kinds of drugs such as calcium (Ca^{2+}) channel antagonists (nifedipine, etc.), antiepileptics (phenytoin, etc.) and immunosuppressants (cyclosporin A, etc.) cause gingival overgrowth as a side effect. However, neither the mechanism of the gingival overgrowth caused by the drugs nor the best method for etiologic therapy of it is yet known. Discovery of a new etiologic drug is urgently needed. Earlier, we claimed that drug-induced gingival overgrowth is caused by a rise in the intracellular Ca^{2+} concentration (Hattori, 2003), increased production of type I collagen, and the release of basic fibroblast growth factor (bFGF), which cytokine causes the proliferation of fibroblasts (Hattori et al., 2004).

Tranilast is a drug known to block the release of chemical mediators and to suppress the proliferation of various cells (Noguchi et al., 1996). We believe that this drug would be the most suitable as a therapeutic agent for gingival overgrowth. Thus, we examined whether or not tranilast would inhibit nifedipine-induced fibroblast proliferation and if so, by what mechanism. The findings obtained suggest that tranilast inhibited the proliferation by depressing the release of bFGF from the gingival fibroblasts.

2. Materials and methods

2.1. Cell culture

Normal human gingival fibroblast Gin-1 cells obtained from Dainippon Pharmaceutic (Japan) were used in these experiments. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The medium also contained antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin, Sigma, USA), and it was changed at intervals of 2 or 3 days.

2.2. Fibroblast proliferation

Nifedipine-induced fibroblast proliferation in the presence and absence of tranilast was examined by using the

* Corresponding author. Tel.: +81 263 52 3100; fax: +81 263 53 3456.
E-mail address: hattori@po.mdu.ac.jp (T. Hattori).

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doi:10.1016/j.ejphar.2004.07.061

reagent water-soluble tetrazolium-1, which is supplied in a commercially available assay kit (Dojindo Laboratories, Japan). The cells were cultured in 96-well microculture plates, with DMEM containing 10% fetal bovine serum used as the medium. Control wells contained only nifedipine (10 μM), whereas tranilast (100 μM) along with the nifedipine was added to the test wells (Nie et al., 1996). Both nifedipine and tranilast were dissolved in dimethyl sulfoxide (DMSO) as a solvent and the final concentration of DMSO in each well did not exceed 1%. The number of cells in a well was 1×10^4 at the starting point. The cell proliferation of fibroblasts with or without tranilast was examined over a time course of 14 days. Briefly, at selected times during the culture period, tetrazolium-1 (10 μl) was added to each well and the plate was then incubated at 37 °C for 2 h (Daniels et al., 2003). The optical density was read with a microplate reader (Model 550, BioRad Laboratories, USA) at 450 nm as a test wavelength and 655 nm as a reference one according to manufacturer's instructions. Assays of both the control group and the test one were performed by using 8 wells for each. Cell numbers were calculated by reference to tetrazolium-1 values obtained beforehand for a known number of viable Gin-1 cells.

2.3. bFGF determination

For this determination the fibroblasts were grown in 35-mm diameter culture dishes. The level of bFGF in the cell-free supernatant of each dish was determined with an enzyme-linked immunosorbent assay kit purchased from R&D Systems (USA) and used according to the manufacturer's instructions (Carroll and Koch, 2003). DMSO (1%) was added to control dishes. The test group was treated with tranilast (1, 10 and 100 μM) for 2 days. A total number of 1×10^5 cells was seeded into each dish containing 1.2 ml medium. The sensitivity of the bFGF assay kit was 3 pg/ml. Assay results were measured by use of the microplate reader, with 450 nm as a test wavelength and 570 nm as the reference one. Assays of both control and test groups were performed.

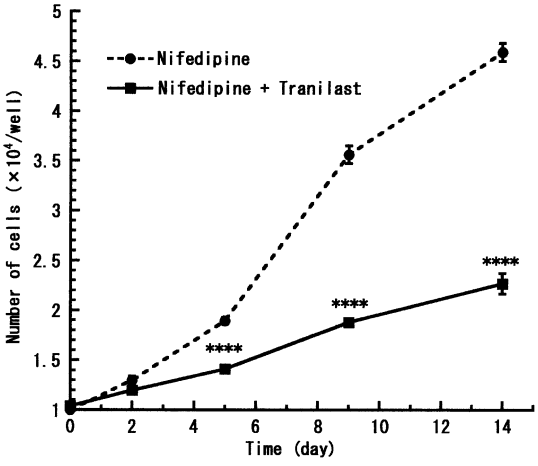

Fig. 1. Effect of tranilast on the nifedipine-induced proliferation of gingival fibroblasts. Tranilast (100 μM) significantly inhibited the nifedipine (10 μM)-induced proliferation. ****P<0.001, N=8 for each time point.

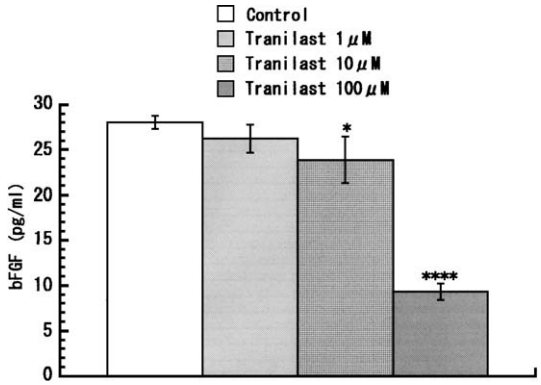

Fig. 2. Effects of tranilast on the release of bFGF from the gingival fibroblasts. Tranilast at concentration of 10 μM and above significantly reduced the level of bFGF in the culture medium. *P<0.05, ***P<0.001, N=8 for each column.

2.4. Chemicals

Tranilast (RIZABEN®) was a generous gift from Kissei Pharmaceutical (Japan). Nifedipine was obtained from Sigma. Other chemicals were from Nacalai Tesque (Japan).

2.5. Statistical analyses

Data were presented as the mean values±the S.E.M. and the number of wells (N). Statistical analysis of the data was performed by using Student's two-sided simple t-test. Differences between mean values were considered significant if the probability of error (P) was less than 0.05.

3. Results

Fig. 1 illustrates the effect of tranilast on the nifedipine-induced proliferation of gingival fibroblasts. When added together with nifedipine, tranilast (100 μM) significantly inhibited the nifedipine (10 μM)-induced proliferation starting on the fifth day of the culture period. It was ensured that the solvent used for nifedipine and tranilast, DMSO (1%), had no effect on the normal proliferation.

Fig. 2 shows the effects of tranilast on the release of bFGF from the cells. Tranilast concentration-dependently reduced the level of bFGF in the culture medium. In the case of tranilast at the concentration above 10 μM, there was significant difference between the control and test groups.

4. Discussion

Tranilast inhibits the proliferation of various fibroblasts, for example, mouse gastric fibroblasts (Yashiro et al., 1997),

human pterygium-derived fibroblasts (Isaji et al., 2000), rabbit dermal fibroblasts (Yasukawa et al., 2002) and a fibrous tissue in oral squamous cell carcinomas in mice (Noguchi et al., 1996). However, tranilast has never been applied to gingival overgrowth. In this present study, we discovered that tranilast suppressed the nifedipine-induced proliferation of gingival fibroblasts (Fig. 1). The reports mentioned above support our observation.

To elucidate the mechanism of inhibition of the proliferation, we examined the effect of tranilast on the release of bFGF from the fibroblasts. Tranilast reduced the bFGF level in the medium (Fig. 2). Since tranilast is an anti-allergic agent that inhibits the release of chemical mediators from mast cells (Azuma et al., 1976), this result may not be surprising. In addition, Adachi et al. (1999) observed that tranilast decreased the serum bFGF level in rats. Moreover, there is a report that tranilast decreases the cytosolic Ca^{2+} concentration. Nie et al. (1996) observed that tranilast attenuated Ca^{2+} entry induced by platelet-derived growth factor in vascular smooth muscle cells of the rat. Thus, we propose that this characteristic cytosolic Ca^{2+}-reducing effect of tranilast is involved in the depression of exocytosis of bFGF.

Since bFGF combines with receptor tyrosine kinase (RTK), it is suggested that bFGF released from the cell membrane stimulates RTKs in an autocrine and/or paracrine manner. As a general mechanism underlying the action of growth factors, Munaron (2002) described that growth factors, by binding to their surface receptors, induce dimerization of RTKs and activation of their tyrosine kinase activity. A second step is the crossphosphorylation of RTKs, with the subsequent recruitment and the activation of intracellular proteins, which then transmit the signal to a plethora of targets, including nuclear transcription factors. Among these signaling molecules, phospholipase C, phosphatidylinositol-3-kinase, *ras*-activating protein, *ras*, mitogen-activated protein kinase and phospholipase A_2 are the most commonly involved. Therefore, we believe that gingival overgrowth may occur as follows: At first, L-type Ca^{2+} channel blockers elevate the cytosolic Ca^{2+} concentration, and then cytosolic Ca^{2+} facilitates the release of bFGF. Next bFGF activates tyrosine kinase, and thereafter gene expression for cell proliferation and synthesis of type I collagen is evoked (Hattori et al., 2004). Consequently, although we did not measure the intracellular Ca^{2+} level, we suspect that tranilast decreases the cytosolic Ca^{2+} concentration and thereby depresses the bFGF release, which depression in turn suppresses the proliferation.

Tranilast has other important properties. There are some reports describing that tranilast inhibits collagen deposition. That is to say, Oshima et al. (2000) reported that tranilast inhibited the proliferation of rabbit fibroblasts by reducing collagen synthesis in these cells. Also, Isaji et al. (1994) described that tranilast inhibited collagen accumulation in hypersensitive granulomatous inflammation in rats. On the other hand, Yasunami et al. (1997) observed that tranilast had an anti-angiogenic effect, which would be favorable for inhibition of cell proliferation. These characteristics of tranilast would seem likely to play a role in depression of gingival overgrowth in vitro.

In conclusion, the present findings demonstrate that tranilast inhibits the nifedipine-induced proliferation of gingival fibroblasts by inhibiting the release of bFGF. Administration of tranilast may thus be clinically effective for the treatment of drug-induced gingival overgrowth.

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