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PROLIFERATION OF CULTURED HUMAN GINGIVAL FIBROBLASTS CAUSED BY ISRADIPINE, A DIHYDROPYRIDINE-DERIVATIVE CALCIUM ANTAGONIST

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Abstract: As it was reported earlier that isradipine, a Ca^{2+} antagonist of dihydropyridine derivative class, caused regression of nifedipine-induced hyperplasia of human gingiva, experiments were performed to examine whether or not isradipine would solely inhibit the proliferation of cultured gingival fibroblasts. Normal human gingival fibroblast Gin-1 cells were used to test the impact of this medication. Fibroblast proliferation in the presence of isradipine (10 μM) was examined by using the reagent water-soluble tetrazolium-1 (WST-1). The level of basic fibroblast growth factor (bFGF) in the cell-free supernatant of each well was determined by using an enzyme-linked immunosorvent assay (ELISA) kit. The production of type I collagen was assayed by ELISA. Isradipine significantly enhanced the cell proliferation from the second day of the culture period. Also, isradipine raised the level of bFGF in the culture medium. The same concentration, also significantly enhanced the production of type I collagen. In conclusion, we were able to prove that isradipine causes the proliferation of cultured gingival fibroblasts as well as other dihydropyridine-derivative Ca^{2+} antagonists do. In order to prevent the gingival overgrowth, it is advisable to be very careful in the use of isradipine as a therapy for hypertension and other indications.

Key words: isradipine; gingival fibroblast; proliferation; bFGF; type I collagen

INTRODUCTION

Nifedipine, a calcium (Ca^{2+}) antagonist of the dihydropyridine (DHP)-derivative class, is frequently used when treating patients suffering from hypertension. Although it is well known that nifedipine generally triggers gingival overgrowth as a side effect, it was clinically reported by Westbrook et al. [15] that isradipine (another DHP-derivative Ca^{2+} antagonist) causes regression of nifedipine-induced hyperplasia of human gingiva. They described that 60 % of patients treated with isradipine exhibited a decrease in gingival hyperplasia, while 66 % of patients treated with nifedipine showed an increase in hyperplasia; a significant difference could be established with $P < 0.05$.

We previously observed that isradipine raised the intracellular calcium concentration ($[Ca^{2+}]i$) by enhancing Ca^{2+} influx through the plasma membrane as well as the release of Ca^{2+} from intracellular Ca^{2+} storage sites [4]. This observation suggests that isradipine exerts its action as a cell proliferation factor that raises the $[Ca^{2+}]i$. Ca^{2+} influx is known to play a key role in the regulation of important intracellular events, such as the control of proliferation of fibroblasts [12]. Thus, we performed experiments to examine whether or not isradipine would inhibit the proliferation of cultured normal human gingival fibroblasts. The results obtained suggest that isradipine enhances their proliferation.

MATERIALS AND METHODS

Normal human gingival fibroblast Gin-1 cells obtained from Dainippon Pharmaceutic Co. Ltd. (Japan) were used for this study.

Fibroblast proliferation in the presence of isradipine (10μM) was examined by using the reagent water-soluble tetrazolium-1 (WST-1; Dojindo Laboratories, Japan), which is supplied in a commercially available assay kit. The concentration of isradipine adopted here was 10 μM, because this concentration significantly blocks Ca^{2+} channels in neurons [7]. The solvent used, ethanol (1 %) shows no effect on fibroblast growth. The cells were cultured in a humidified 5 % CO_2 atmosphere in 96-well microculture plates for 12 days, with Dulbecco's modified Eagle's medium containing 10 % fetal bovine serum which was added to the medium. Control fibroblasts only contained the medium (1×10^4/well), whereas isradipine (10 μM) was added to the test wells. The cell proliferation of fibroblasts, incubated with or without isradipine, was examined over a period of 12 days. At selected times during the culture period, WST-1 (10 μl) was added to each well, and the plate was then incubated at 37 °C for 2 hours [3]. According to manufacturer's instructions the optical density was determined with a microplate reader (Model 550, BioRad Laboratories, USA) at 450 nm which serve as a test wavelength and 655 nm as a reference [5]. Assays of both, the control group and the test fibroblasts, were performed by using 8 wells for each study. The cell numbers were calculated by reference to WST-1 values obtained from a known number of viable Gin-1 cells prior to the study.

The level of the initial fibroblast growth factor (bFGF) in the cell-free supernatant of each well was determined by using an enzyme-linked immunosorvent assay (ELISA) kit purchased from R & D Systems (USA) according to the manufacturer's instructions [2]. Fibroblasts were seeded into a 96-well microculture plate at a density of 1×10^4/well. The

sensitivity of the bFGF assay kit extended as far as 3 pg/ml. Assays were measured by 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.

The production of type I collagen was assayed by ELISA (Chondrex, USA) according to the manufacturer's instructions [10]. The cells were cultured in 96-well microculture plates. Control fibroblasts only contained the medium (1 x 10^4/well). Values were measured with the microplate reader. The optical densities were determined at 490 nm. The conditions for cell culture studies and the numbers of assays of both, control wells and test ones, were the same as described above.

Isradipine was a free sample from Novartis Pharma (Switzerland). Tissue culture reagents were purchased from Gibco (USA). All other chemicals were from Nacalai Tesque (Japan).

Data were represented as the mean value ± the standard error of the mean and the number of cells (N). Statistical analysis of the data was performed by using Student's 2-sided simple *t*-test. Differences between mean values were considered significant if the probability of error (P) was less than 0.05.

Results

This study showed that the calcium anagonist isradipine had an influence on the proliferation rate of cultured gingival fibroblasts. Fig. 1 illustrates the effect of isradipine on the cell proliferation up to day 12. Contrary to our expectation, isradipine (10 μM) significantly enhanced the cell proliferation starting on the second day of the incubation period. Finally, isradipine increased the cell proliferation which results in a value approx. 120 % of the control.

Fig. 2 shows the effect of isradipine on the release of bFGF from the cells. Isradipine (10 μM) significantly raised the level of bFGF in the culture medium.

Fig. 3 illustrates the effect of isradipine on the production of type I collagen. Isradipine (10 μM) also significantly enhanced the production of type I collagen. The administration of isradipine thus increased the level of type I collagen in the medium by almost 55 ng/ml.

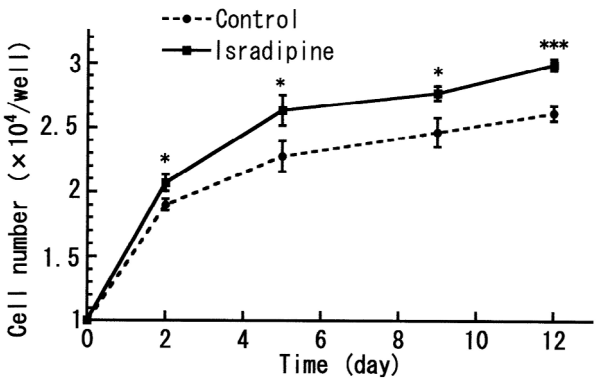

Fig. 1. Effect of isradipine on Gin-1 cell proliferation. Isradipine (10 μM) significantly enhanced the proliferation starting on the second day of the incubation period. *P<0.05, ***P<0.005, N = 8 for each time point.

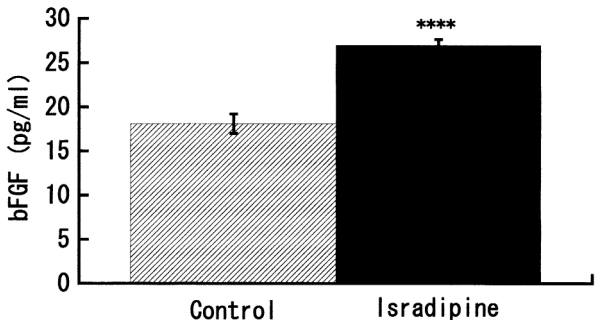

Fig. 2. Effect of isradipine on bFGF release from the cells. Isradipine (10 μM) significantly raised the level of bFGF in the culture medium. ****P<0.001, N = 8 for each group.

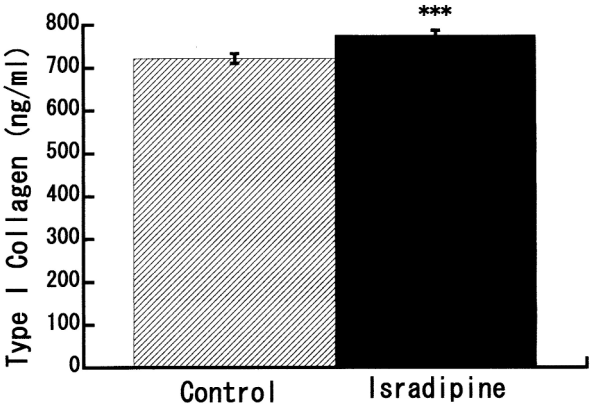

Fig. 3. Effect of isradipine on the production of type I collagen. Isradipine (10 μM) also significantly enhanced the production of type I collagen. ***P<0.005, N = 8 in each group.

Discussion

There are many reports indicating that isradipine inhibits the activity of numerous sensitive cells, such as neurons, smooth muscle cells, and cells of cardiovascular system, by blocking the Ca^{2+} influx through the Ca^{2+} channels in such cells [6, 7, 9]. Jensen et al. [7] described that, as an L-type voltage-dependent Ca^{2+} channel antagonist, isradipine (5 μM) inhibited the posttetanic potentiation in cultured rat hippocampal neurons with a probability of 65 %.

There are few reports concerning the relationship between isradipine and gingival fibroblasts [15]. In the present study, isradipine enhanced the proliferation of these cells. This is the first report of such an effect on cultured gingival fibroblasts. The mechanism of drug-induced gingival overgrowth may include the stimulation of aid bacteria, because signs of inflammation play a vital part in this phenomenon [1]. However, our study indicates that isradipine causes gingival overgrowth on the one hand by a raised growth factor (bFGF) and on the other hand by increasing the extracellular matrix (collagen) production which causes a proliferation of gingival fibroblasts.

Our present results show that isradipine increases the level of bFGF. Saito et al. [13] immunohistochemically investigated the effect of nifedipine and

phenytoin on human gingiva tissue. The authors demonstrated an increased synthesis of bFGF and their receptors caused by nifedipine and showed that this increase is related to the pathogenesis of drug-induced gingival hyperplasia. Considering the fact that Ca^{2+} antagonists raise the bFGF level, their report supports our observation. Since bFGF combines with the receptor tyrosine kinase (RTK), it is suggested that bFGF released from the cell membrane stimulates RTKs in an autocrine and/or paracrine manner [11].

An increase in the collagen content is observed in drug-induced gingival overgrowth [14]. Kataoka et al. [8] reported that a decrease in collagen degradation due to lower phagocytosis is closely associated with an increase in Type I collagen accumulation in nifedipine-treated rat gingiva. Referring to our result that isradipine facilitated the production of collagen, it seems likely that isradipine may be responsible for the gingival overgrowth.

bFGF is a typical angiogenesis accelerator. Zhu [16] concluded that subepicardial administration of bFGF in slow-release microcapsules in the infarcted rabbit results in an effective angiogenesis. Thus, we consider that this characteristic of bFGF is involved in the gingival overgrowth.

As a general mechanism underlining the action of growth factors, Munaron [11] 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 phosphsolipase A_2 are the most commonly involved. Therefore, about the mechanism by which isradipine causes gingival overgrowth, we believe as follows: At first, isradipine elevates the $[Ca^{2+}]i$ [4] and then, Ca^{2+} releases bFGF, next bFGF activates tyrosine kinase, and thereafter, evokes gene expressions for cell proliferation and synthesis of type I collagen.

In conclusion, we confirm that isradipine causes the proliferation of gingival fibroblasts as well as other DHP-derivative Ca^{2+} antagonists do. In order to prevent the gingival overgrowth, it is indispensable to be careful in the use of isradipine as a therapy of hypertension and other indications.

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