Present research status on drug-induced gingival overgrowth

A possible therapeutic for gingival overgrowth caused by calcium channel blockers

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Abstract: In the present study, the results of characters in nifedipine responders (NIFr) and nifedipine non-responders (NIFn) are summarized, and the possibility of using tenidap and 18α-glycyrrhetinic acid (18α-GA) as a therapeutic for gingival overgrowth caused by calcium channel blockers is investigated. 18α-GA inhibited cell proliferation and G1/S transition were induced in NIFr cells. It was also shown that cell cycle control proteins were down-stream targets in the growth-inhibition activity of 18α-GA in NIFr cells. Tenidap discharges intracellular Ca2+ store, resulting in a depletion of intracellular Ca2+ store in NIFr cells. It also inhibits cell growth, DNA and collagen syntheses, lowered intracellular pH, and enhanced matrix metalloproteinase-1 formation in NIFr cells. These results suggest that 18α-GA and tenidap might be effective for the prevention of gingival overgrowth caused by calcium channel blockers.

Key words: nifedipine, tenidap, 18α-glycyrrhetinic acid, gingival fibroblasts, cell cycle

Introduction

Gingival overgrowth in response to anti-epileptics (phenytoin), immunosuppressants (cyclosporin A), and calcium channel blockers (nifedipine, diltiazem, verapamil, nicardipine) is well recognized. Particularly, many case reports have implicated nifedipine, one of the dihydropyridine calcium channel blockers, as a cause of gingival overgrowth (first reported by Ramon, et al.1 and Lederman, et al.2). The incidence of gingival overgrowth due to nifedipine has been reported to be 6.5%3, more than 10%4, 13.7%5, 15%6, and 20%7. However, the mechanism of the nifedipine-induced gingival overgrowth has not been fully clarified. We have previously demonstrated differences in cell growth, calcium response, intracellular crosstalk, and cell cycle between gingival fibroblasts derived from a nifedipine-reactive patient (nifedipine respond-er, NIFr) and those from a nifedipine-nonreactive patient (nifedipine non-responder, NIFn).

In this review, the results of characters in NIFr and NIFn are summarized, and the possibility of using tenidap and 18α-glycyrrhetinic acid (18α-GA) as a therapeutic for gingival overgrowth caused by calcium channel blockers is investigated.

Characterization of NIFr and NIFn

1. Response to calcium channel blockers on proliferation, DNA and collagen syntheses

NIFr cells exhibited greater proliferation rates and DNA and collagen syntheses than NIFn cells in the presence of 1 μM of calcium channel blockers (nifedipine, diltiazem, nicardipine, and verapamil) or phenytoin8. Therefore, it is possible that fibroblasts from reactive patients may also be susceptible to the other calcium channel blockers, which indicates that those

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patients who develop gingival overgrowth because of nifedipine medication may also develop it in response to other calcium channel blockers.

2. Response to stimulants on intracellular free Ca\(^{2+}\) concentration

NIFn cells showed a greater cytosolic calcium response to bradykinin, thrombin, prostaglandins E\(_2\) and F\(_{2\alpha}\) and platelet derived growth factor-BB than NIFr cells. On the contrary, NIFr cells responded more intensively to histamine and bombesin. Among those stimulants, bradykinin induced the greatest response in both NIFr cells and NIFn cells\(^9\).

3. Response to basic fibroblast growth factor on cell growth and cell cycle regulators

We compared the differences in cell proliferation, cell cycle, and expression of cell cycle regulating proteins in the presence of basic fibroblast growth factor (bFGF) observed between NIFr and NIFn cells.

The proliferation rate of NIFr cells in the presence of bFGF was significantly higher than in NIFn cells. The proportion of NIFr cells that underwent progression to the S and G\(_2/M\) phases from the G\(_0/G_1\)
phase in the presence of bFGF was greater than that of NIFn cells, and also the expressions of mRNAs for cyclins A, B, D1 and E, and cyclin-dependent kinases (CDKs) 1, 2, 4, and 6 were greater in NIFr cells than NIFn cells in the presence of bFGF\(^\text{10}\). Increases of phosphor-retinoblastoma (pRB) (Ser807/811), pCDK2 (Thr160), CDK2, and cyclin E protein levels in NIFr cells were greater than that of those in NIFn cells. The elevations of pRB (Ser780), RB, and cyclin A protein levels in NIFr cells did not differ from those of NIFn cells. The growth of NIFr cells was greater than that of NIFn cells as a result of the active G\(_1\)/S transition of NIFr cells, by the increments of cyclin E, pCDK2 and pRB (ser807/811) protein in NIFr cells\(^{11}\). NIFr may be more susceptible to the growth factors in fetal calf serum as well as bFGF resulting in increased cyclins and CDKs than NIFn.

**4. Intracellular Crosstalk**

A possible role of nifedipine in gingival overgrowth is summarized in Fig. 1\(^{11}\). Nifedipine inhibits phosphodiesterase to increase protein kinase G, and then activates p38 mitogen-activated protein kinase (MAPK) and activating transcription factor-2, resulting in apoptosis and inhibition of cell growth. As a result, NIFn cells were more active than NIFr cells, indicating that NIFn cell growth was depressed by nifedipine, which also induced NIFn cells to undergo apoptosis\(^{12}\). Nifedipine accelerates phospholipase D activity to produce diacylglycerol from phosphatidic acid and phosphatidylcholine, which then activates PKC, resulting in the cell growth and cell cycle transition\(^{13}\). bFGF accelerates protein kinase C (PKC) activity through phospholipase C\(_\gamma\). A further investigation should be done to clarify the intermediate pathways.

**Prevention of Gingival Overgrowth**

1. **Application of tenidap**

Tenidap, (±)-5-chloro-2,3-dihydro-3-(hydroxy-2-thienylmethylene)-2-oxo-1H-indole-1-carboxamide, is a new anti-inflammatory agent, which has been shown to inhibit IgE-mediated N-acetylglucosaminidase secretion from mast cells\(^{14}\), release activated collagenase from neutrophils\(^{15}\), inhibit leukotriene B\(_4\) and prostanoid synthases in human neutrophils\(^{16}\), form 5-lipoxygenase products in human subjects\(^{17}\), inhibit the production of interleukin-1, 6 and tumor necrosis factor from human Hep3B hepatoma cells\(^{18}\), and inhibit the antigen-induced increase in intracellular Ca\(^{2+}\) and also both antigen- and thapsigargin-induced Ca\(^{2+}\) influx across the plasma membrane in a mast cell line\(^{19}\).

We investigated the effect of tenidap on intracellular free Ca\(^{2+}\) concentration, DNA synthesis by means of \(^{3}\text{H}\) thymidine incorporation, collagen synthesis by means of \(^{3}\text{H}\) proline incorporation, cell proliferation, and intracellular pH in nicardipine-reactive human gingival fibroblasts. Tenidap discharges intracellular Ca\(^{2+}\) store, resulting in a depletion of intracellular Ca\(^{2+}\) store and also tends to inhibit Ca\(^{2+}\) influx in gingival fibroblasts\(^{20}\). Tenidap inhibited \(^{3}\text{H}\) thymidine and \(^{3}\text{H}\) proline incorporation, and lowered intracellular pH\(^{21}\). Tenidap enhanced intra- and extra-cellular matrix metalloproteinase-1 (MMP-1) concentrations and MMP-1 mRNA expression in NIFr cells. However, PKC inhibitor (bisindolylmaleimide), MAPK kinase (MEK) 1/2 inhibitor (U0126) and P38 MAPK inhibitor (SB203580) did not inhibit MMP-1 mRNA expression enhanced by tenidap in NIFr. Tenidap enhanced phosphorylated extracellular signal-regulated kinase (ERK) 1 and 2\(^{22}\).

Consequently, the present in vitro data suggest that tenidap significantly inhibits DNA and collagen synthesis at a concentration of greater than 20μM (6.85μg/mL). In the first phase of the clinical trial, although plasma C\(_\text{max}\) was 8.305, 17.006, and 21.009μg/mL after a single oral dose of 40, 80, and 120mg tenidap, respectively (unpublished data, Pfizer Pharmaceutical Co. Ltd.), more than 99% of tenidap bound to plasma protein. Cleveland, et al\(^{10}\) also indicated that the plasma drug level at therapeutic doses in arthritis patients reaches 60μM (20.6μg/mL), but tenidap is substantially bound by serum albumin. The distribution of tenidap to oral tissue is hardly available. In case of rats, the distribution to salivary gland is 17.4 to 19.7% (unpublished data, Pfizer Pharmaceutical Co. Ltd.). Thus, it is estimated that a sufficient concentration of tenidap might not be able to reach the oral tissue, which is enough to reduce DNA and collagen syntheses in gingiva by a systemic tenidap administration. In our preliminary experiment using rats\(^{23}\), the local application of a high dose of tenidap effectively pre-
vented gingival overgrowth caused by calcium channel blockers, especially nifedipine. Therefore, tenidap may be one of the drugs that prevents gingival overgrowth. Thus, the continuous retention of tenidap in local areas, such as in periodontal pockets, might affect gingival fibroblasts and reduce their growth through apoptosis.

2. Application of 18α-glycyrrhetinic acid

Licorice has been used in cough preparations as well as sweetening agents in food products. It also has ulcer-healing properties and mild anti-inflammatory effect. The major water-soluble constituent of licorice is glycyrrhizin, which is known to be partly hydrolyzed by glucononidase to its aglycone glycyrrhetinic acid which exists in 18 alpha (18α- GA) and 18 beta stereoisomeric forms. 18α- GA has a variety of interesting activities such as the growth-promoting effect of hepatocyte, an anticancer effect, an anti-inflammatory effect, and an inhibitory effect on cell proliferation.

It has been reported that 18α- GA induces the growth of primary cultured adult rat hepatocytes, down-regulates the production of inflammatory chemokine eotaxin 1 in a human lung fibroblast cell line, and inhibits cell growth in MCF-7 cells and in skin tumors. 18α- GA was a good anti-proliferative agent especially on those cells whose replication rate was slow, by inhibiting the onset of progression. Since the growth rate of NIFr cells was greater than that of NIFn cells, it might be interesting to clarify if 18α- GA has an activity on the cell growth of NIFr cells.

18α- GA inhibited cell proliferation and G1/S transition induced by bFGF. It was also shown that cell cycle control proteins, such as pRB (ser780), pRB (ser807/811), CDK4, CDK6, CDK2, cyclin D1, and cyclin A, were downstream targets in the growth-inhibition activity of 18α- GA in NIFr cells. In the development of nifedipine-induced gingival overgrowth, the inflammation and cell growth of NIFr cells are important factors. Based on these findings, 18α- GA, which has an anti-inflammatory effect and inhibits growth of NIFr cells, may have a positive role in nifedipine-induced gingival overgrowth therapy.

References


薬物性歯肉増殖症に関する研究の現状

カルシウム拮抗薬による歯肉増殖症における薬物治療の可能性

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本研究ではニフェジピン感受性（NIFr）および非感受性（NIFn）ヒト歯肉培養線維芽細胞の特徴をまとめると共にtenidayと18α-glycyrrhetinic acid (18α-GA)のニフェジピンによる歯肉増殖症の薬物治療薬としての可能性を探った。NIFrにおいて、18α-GAは細胞増殖やG0/G1 phase からS phaseへの移行を抑制した。さらに、18α-GAの増殖抑制活性において細胞周期制御タンパクがdown-stream target となっていることが示された。tenidayはNIFrにおいて、細胞内Ca²⁺ storeを枯渇し細胞外からのCa²⁺ influxを阻害する。さらに、細胞増殖能、DNA合成能、collagen合成能を抑制し、pHiを低下させ、matrix metalloproteinase-1産生を増加することなどを明らかにしている。以上の結果から、tenidayと18α-GAはカルシウム拮抗薬による歯肉増殖症の治療に有用である可能性が示唆された。

キーワード：ニフェジピン, テニダップ, 18α-グリチルレチン酸, 歯肉由来線維芽細胞, セルサイクル