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REVIEW ARTICLE

Drug-induced gingival overgrowth and its tentative pharmacotherapy

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Summary The characters in gingival fibroblasts derived from a nifedipine-reactive patient (nifedipine responder: NIFr) and those from a nifedipine-nonreactive patient (nifedipine non-responder: NIFn) are summarized, and investigated a possibility of tenidap, (±)-5-chloro-2,3-dihydro-3-(hydroxy-2-thienylmethylene)-2-oxo-1H-indole-1-carboxamide, and 18α-glycyrrhetinic acid (18α-GA) as a therapeutics for gingival overgrowth caused by calcium channel blockers. Tenidap discharges intracellular Ca^{2+} store, resulting in a depletion of intracellular Ca^{2+} store in cultured human gingival fibroblasts. It also inhibited cell growth, DNA and collagen syntheses, lowered intracellular pH in nicardipine responder cells, and enhanced matrix metalloproteinase-1 formation in NIFr cells. 18α-GA inhibited cell proliferation and G_1/S transition 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. These results suggest that tenidap and 18α-GA might be effective for the prevention of gingival overgrowth caused by calcium channel blockers.

Contents

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1. Introduction

Gingival overgrowth in response to anti-epileptics (phenytoin and sodium valproate), immunosuppressants (cyclosporine A), and calcium channel blockers (nifedipine, diltiazem, verapamil, nicardipine, amlodipine, manidipine, and nisoldipine) is well-recognized. Particularly, many case reports have implicated nifedipine (NIF), 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 NIF has been reported to be 6.5% [3], 7.6% [4], more than 10% [5], 11.6% [6], 15% [7], and 20% [8]. We also reported the incidences of gingival overgrowth caused by amlodipine, diltiazem, manidipine, nicardipine, and nisoldipine were 1.1%, 4.1%, 1.8%, 0.5%, and 1.1%, respectively [4]. However, the mechanism of the NIF-induced gingival overgrowth has not been well clarified. We have previously demonstrated the difference on cell growth, collagen synthesis, calcium response, intracellular crosstalk, and cell cycle between gingival fibroblasts derived from a NIF-reactive patient (NIF responder, NIFr) and those from a NIF-nonreactive patient (NIF non-responder, NIFn).

In this review, the specific characters in NIFr and NIFn are summarized, and a possibility of tenidap, (±)-5-chloro-2,3-dihydro-3-(hydroxy-2-thienylmethylene)-2-oxo-1H-indole-1-carboxamide, and 18α-glycyrrhetinic acid (18α-GA) was investigated as a therapeutics for gingival overgrowth caused by calcium channel blockers.

2. Characterize NIFr and NIFn cells

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

In general, the histological examination of the gingival specimens from patients reactive to NIF medication revealed large bundles of dense collagenous fivers with a moderate increase of fibroblasts in addition to epithelial hyperplasia with acanthosis and parakeratosis, and elongation of the rete pegs. Thus, it was thought interesting to focus on gingival fibroblasts obtained from NIFr and NIFn. As the first step of the investigation, the difference between NIFr cells and NIFn cells was studied. 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 phenytoin [9]. Therefore, it is possible that gingival fibroblasts from NIF-reactive patients may be also susceptible to the other calcium channel blockers, which indicates that those patients who developed gingival overgrowth because of NIF medication may also develop it in response to other calcium channel blockers. This was confirmed later, but not for nisoldipine responder cells [10]. Thus, fibroblasts from patients reactive to NIF and nicardipine medication gave a better cell proliferation rate, DNA synthesis, and an increased number of EGF receptors compared to non-drug-treated control, but not in fibroblasts from patients reactive to nisoldipine medication. In general, the presence of tooth and gingival crevice are essential to generate drug-induced gingival overgrowth, suggesting that the presence of gingival crevicular fluid might be important for gingival overgrowth. Interleukin-1α (IL-1α) predominates in gingival crevicular fluid and greater amount of NIF is found in gingival crevicular fluid in the patients with periodontal disease, it was interesting to investigate the simultaneous effect of NIF and IL-1α on cell proliferation and DNA synthesis. The presence of IL-1α resulted in greater cell proliferation and DNA synthesis than in the presence of NIF alone and NIFr cells showed greater response. The DNA synthesis rate with a combination of NIF and IL-1α was also higher than that for NIF or IL-1α alone. Thus, the interaction between NIF and gingival inflammation might play an important role in the pathogenesis of NIF-induced gingival overgrowth [11]. IL-1α also yielded significantly higher basic fibroblast growth factor (bFGF) production and release, and also enhanced bFGF mRNA expression. In addition, levels of released bFGF were significantly higher in cells pretreated with IL-1α, followed by bradykinin and thapsigargin in the presence of extracellular Ca^{2+}. The transient mobilization of intracellular Ca^{2+} accelerated the release of bFGF in IL-1α-pretreated cells, but not in untreated cells [12]. These findings suggest that NIFr cells are more sensitive to NIF and that IL-1α accelerate this sensitivity through bFGF formation.

2.2. Response to stimulants on intracellular free Ca^{2+} concentration

The cell proliferation in cultured fibroblasts involves a sequence of biochemical events, which begin in part at the cell surface by mitogen stimulation and progress temporally and spatially to the cell nucleus through signal transduction pathways. Among the earliest of these events are dramatic changes in intracellular free Ca^{2+} concentration in a variety of cell types via a direct effect of inositol 1,4,5-trisphosphate (IP_3) on the ligand-activated calcium channels in intracellular Ca^{2+}-storing organelles [13,14]. Therefore, it was interesting to investigate if there are any differences between NIFr cells and NIFn cells against the stimulants, such as bradykinin, thrombin, histamine, bombesin, prostaglandins E_2 and $F_{2\alpha}$, and platelet derived growth factor-BB. 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 than NIFn cells. Among of those stimulants, bradykinin introduced the greatest response in both NIFr cells and NIFn cells [15]. NIF (1 μM) reduced nearly 20% of the intracellular Ca^{2+} concentration response stimulated by bradykinin in both NIFr and NIFn cells in the absence of extracellular Ca^{2+}. Since gingival fibroblasts used in the present study do not possess L-channel, the target Ca^{2+} channel for calcium channel blockers, the inhibition of intracellular Ca^{2+} concentration response by NIF might be another pharmacological effect of NIF.

2.3. Response to growth factors on cell growth and cell cycle regulators

The growth factor, such as bFGF and insulin-like growth factor-I (IGF-I), is potent mitogen for fibroblasts that promote cell cycle progression. Both bFGF and IGF-I are important mediators in the development of gingival fibroblasts. We assumed that there might be some differences between NIFr and NIFn cells in response to bFGF and IGF-I. Thus, we compared the differences in cell proliferation, cell cycle, and expression of cell cycle regulating proteins between NIFr and NIFn cells in the presence of bFGF and IGF-I.

The proliferation rate of NIFr cells in the presence of bFGF and IGF-I significantly increased than that of NIFn cells. The proportion of NIFr cells that had undergone progression to the S and G_2/M phases from the G_0/G_1 phase in the presence of bFGF or IGF-I was greater than that of NIFn cells, and also that expressions of mRNAs for cyclins A, B_1, D_1 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 [16,17]. Increases of phosphor-retinoblastoma (pRB) (Ser807/811), pCDK2 (Thr160), CDK2, and cyclin E protein levels in NIFr cells were greater than those in NIFn cells, in the presence of bGFG and IGF-I. In case of bFGF, the elevations of pRB (Ser780), RB, and cyclin A protein levels in NIFr cells did not differ from those of NIFn cells, but pRB(ser780) in case of IGF-I. The growth of NIFr cells was greater than 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 [17,18] (Fig. 1). Therefore, NIFr cells may be more susceptible to the growth factors in fetal calf serum as well as bFGF and IGF-I resulting in increased cyclins and CDKs than NIFn cells.

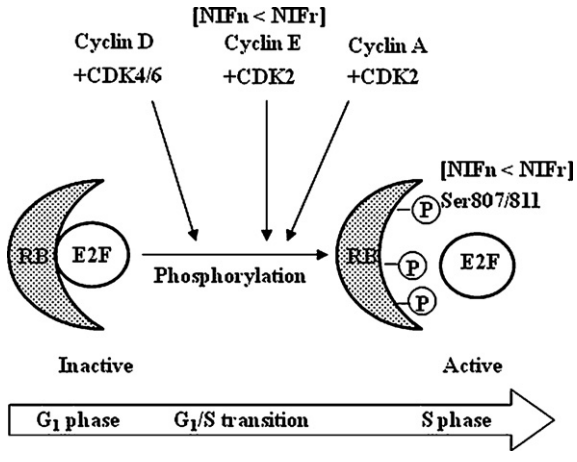

Figure 1 Relationship between RB, RB kinase, and E2F at G_1/S transition. When RB binds E2F at G_1 phase, expression of the target gene is suppressed. At late G_1, RB is phosphorylated forming pRB by cyclin D-CDK4/6, and then by cyclin E-CDK 2 and cyclin A-CDK2, leading to the release of E2F from RB [18]. In NIFr cells, pRB (ser807/811) is phosphorylated through cyclin E-CDK2. RB, retinoblastoma protein; pRB, phosphorylated retinoblastoma protein; E2F, transcription factor E2F; CDK, cyclin-dependent kinase.

2.4. Intracellular crosstalk

A possible role of NIF for gingival overgrowth is summarized in Fig. 2 [18]. NIF inhibits phosphodiesterase to increase protein kinase G, and then activates p38 mitogen-activated protein kinase (p38 MAPK) and activating transcription factor-2, resulting in apoptosis and inhibition of cell growth. Under this circumstance, NIFn cells were more active than NIFr cells, indicating NIFn cell growth was depressed by NIF, which also induced NIFn cells to undergo apoptosis [19]. This was confirmed in NIFr and NIFn cells after stimulation by LPS to find that apoptotic cells in NIFn cells were significantly increased as well as p53 and bcl-2 mRNA expressions compared to NIFr cells [20]. NIF did not alter intracellular IP_3 level or intracellular Ca^{2+} concentration in both NIFr and NIFn cells. However, we found that NIF increased intracellular diacylglycerol (DAG) level and protein kinase C (PKC) activity. This indicates that NIF accelerates phospholipase D activity to produce DAG from phosphatidic acid and phosphatidylcholine, which then activates PKC, resulting in the cell growth and cell cycle transition [21]. bFGF and IGF-I accelerates PKC activity through phospholipase Cγ. The relationship between cAMP level and intracellular Ca^{2+} response elicited by isoprenaline and histamine, respectively, in NIFr and NIFn cells was investigated to find that NIFr cells showed increased intracellular Ca^{2+} and cAMP levels [22]. The increase of intracellular Ca^{2+} level elicited by histamine was depressed by isoprenaline, where NIFn cells responded more than NIFr cells. A further investigation should be done to clarify the intermediate pathways.

3. Prevention of gingival overgrowth

3.1. Application of tenidap

Tenidap is a new anti-inflammatory agent, which has been shown to inhibit IgE-mediated N-acetylglucosaminidase secretion from mast cells [23], release activated collagenase from neutrophils [24], inhibit leukotriene B_4 and prostanoid syntheses in human neutrophils [25], form 5-lipoxygenase products in human subject [26], inhibit production of interleukin-1, 6, and tumor necrosis factor from human Hep3B hepatoma cells [27], 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 [28].

We investigated the effect of tenidap on intracellular free Ca^{2+} concentration in cultured human gingival fibroblasts. Tenidap discharges intracellular Ca^{2+} store, resulting in a depletion of intracellular Ca^{2+} store and that tenidap functions on inhibition of Ca^{2+} influx in gingival fibroblasts [29]. We also studied DNA synthesis by means of [^{3}H]-thymidine incorporation, collagen synthesis by means of [^{3}H]-proline incorporation,

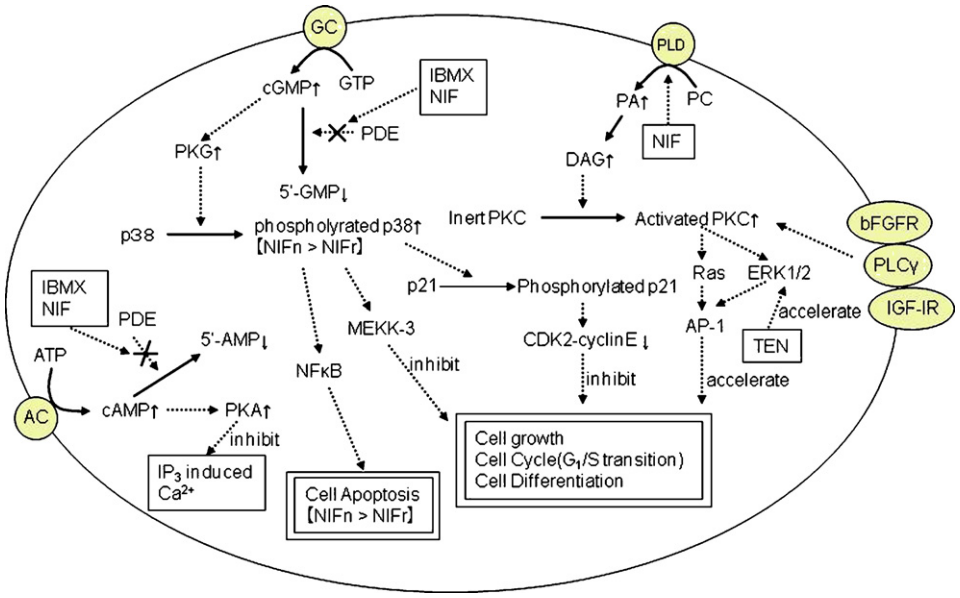

Figure 2 A possible role of nifedipine for gingival overgrowth. Nifedipine inhibits PDE to increase PKG, then activate p38 MAPK resulting in apoptosis and inhibition of cell growth and cell cycle transition (figure modified from Ref. [18]). In this occasion, NIFn cells were more active than NIFr cells, indicating NIFn cells were depressed cell growth by nifedipine, as well as NIFn cells undergo apoptosis by nifedipine. Nifedipine also inhibit PDE to increase PKA which accelerates IP_3 induced intracellular Ca^{2+} concentration and also inhibits Ca^{2+} entry to intracellular Ca^{2+} store via inhibition of Ca^{2+}-ATPase to result in the deprivation of Ca^{2+} in the store. Nifedipine accelerates PLD activity to produce DAG from PA and PC, and then activate PKC, resulting in cell growth, cell cycle transition. Growth factors, such as bFGF and IGF-I, activates PLCγ to activates PKC, resulting in cell growth and cell cycle transition. AP-1, activator protein 1; AC, adenylate cyclase; DAG, diacylglycerol; GC, guanylate cyclase; IBMX, 3-isobutyl-1-methylxanthine; IGF-I, insulin-like growth factor-I; IP_3, inositol-1, 4, 5-trisphosphate; MEKK-3, MAPK/ERK kinase kinase; NIF, nifedipine; p38, p38 mitogen-activated protein kinase (MAPK); PA, phosphatidic acid; PC, phosphatidylcholine; PDE, phosphodiesterase; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PLC, phospholipase C; PLD, phospholipase D.

cell proliferation, and intracellular pH in nicardipine-reactive human gingival fibroblasts. Tenidap inhibited [^{3}H]-thymidine and [^{3}H]-proline incorporation, depressed cell proliferation, and lowered intracellular pH [30]. Tenidap enhanced intra- and extracellular matrix metalloproteinase-1 (MMP-1) concentrations and MMP-1 mRNA expression, and increased phosphorylated extracellular signal-regulated kinase 1 (phospho-ERK1) and phospho-ERK2 in NIFr cells. The previous report that tenidap depressed synthesis of collagen might be explained by the increased formation of MMP-1 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 [31].

Consequently, the present in vitro data suggest that tenidap significantly inhibits DNA and collagen syntheses at a concentration of greater than 20 μM (6.85 μg/mL). In the first phase of the clinical trial, although plasma C_{max} was 8.305, 17.006, and 21.009 μg/mL after a single oral dose of 40, 80, and 120 mg tenidap, respectively (unpublished data, Pfizer Pharmaceutical Co. Ltd.), more than 99% of tenidap bound to plasma protein. Cleveland et al. [28] also indicated that the plasma drug level at therapeutic doses in arthritis patients reaches 60 μM (20.6 μg/mL), but tenidap is substantially bound by serum albumin. Therefore, the distribution of tenidap to oral tissue is hardly available. In case of rats, the distribution to salivary gland is 17.4—19.7% (unpublished data, Pfizer Pharmaceutical Co. Ltd.). Thus, it could be estimated that enough tenidap concentration might not be able to reach to the oral tissue, which is enough to reduce DNA and collagen syntheses in gingival fibroblasts by a systemic tenidap administration. The accumulated collagen fiber in overgrown gingival tissue is also reduced by intra- and extracellular MMP-1 activated by tenidap. In our preliminary experiment using rats, the local application of high dose of tenidap (50 mg/mL, applied into the gingival crevicular region, once a week) might be effective to prevent gingival overgrowth caused by calcium channel blocker, especially NIF [32]. The same trend was also found using dogs. Thus, degree of severity of gingival overgrowth, depth of gingival crevice, and histopathological findings of gingival tissue indicated a significant depression of gingival overgrowth by tenidap [33]. Therefore, tenidap may be one of the drugs that prevent gingival overgrowth. Thus, the continuous retention of tenidap in local area, such as in periodontal pockets, might affect gingival fibroblasts to reduce its growth through apoptosis.

3.2. Application of 18α-glycyrrhetinic acid

Licorice has been used in cough preparations as well as sweetening agent 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 glucuronidase to its aglycone glycyrrhetinic acid which exists in 18 alpha (18α-GA) and 18 beta stereoisomeric forms [34]. 18α-GA has a variety of interesting activities such as the growth-promoting

effect of hepatocyte [35], an anticancer effect [36,37], an anti-inflammatory effect [38,39], and an inhibitory effect on cell proliferation [36]. It has been reported that 18α-GA induces growth of primary cultured adult rat hepatocytes [35], down-regulates production of inflammatory chemokine eotaxin 1 in a human lung fibroblast cell line [38], and inhibits cell growth in MCF-7 cells [36] and in skin tumors [37].

18α-GA was a good anti-proliferative agent especially on those tumor cells whose replication rate was slow, by the inhibition of the onset of progression [36]. 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 G_1/S transition induced by bFGF in NIFr cells [40]. It was also shown that cell cycle control proteins, such as pRB(ser780), pRB(ser807/811), CDK4, CDK6, CDK2, cyclin D_1, cyclin A, were down-stream targets in the growth-inhibition activity of 18α-GA in NIFr cells. In the development of NIF-induced gingival overgrowth, an inflammation and the cell growth of NIFr cells are important factors [9,12,41]. We have recently demonstrated that 18α-GA increased the number of cells that underwent apoptosis and decreased G_0/G_1 phase cells in NIFr. The mRNA expression of bcl-2, the suppressor for apoptosis, was suppressed by 18α-GA in NIFr cells but not in NIFn cells. However, the expression of p53 mRNA was not changed in NIFr cells [42]. In NIFr and NIFn cells, it was demonstrated that apoptotic cells in NIFn cells were significantly increased as well as p53 and bcl-2 mRNA expressions compared to NIFr cells after stimulation by LPS. Thus, 18α-GA induces apoptosis of NIFr cells by inhibiting expression of bcl-2 mRNA. Based on these findings, 18α-GA, which has anti-inflammatory effect and inhibits growth of NIFr cells, may have a positive role in NIF-induced gingival overgrowth therapy.

4. Summary

The characters in NIFr and NIFn cells are compared. NIFn cells tended to undergo apoptosis and increase p53 and bcl-2 mRNA expression in the presence of LPS compared to NIFr cells. NIF might act in the same manner as that of LPS, indicating the growth of NIFn cells was depressed in the presence of NIF. NIFr cells were more susceptible to calcium channel blockers in cell proliferation, DNA and collagen syntheses than NIFn cells. Also NIFr cells were more sensitive to NIF and IL-1α accelerated this sensitivity through bFGF formation.

Tenidap discharged intracellular Ca^{2+} store, resulting in a depletion of intracellular Ca^{2+} store in cultured human gingival fibroblasts. It also inhibited cell growth, DNA and collagen syntheses, lowered intracellular pH in nicardipine responder cells, and enhanced MMP-1 formation in NIFr cells. 18α-GA inhibited cell proliferation and G_1/S transition 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. These results suggest that 18α-GA and tenidap might be effective for the prevention of gingival overgrowth caused by calcium channel blockers.

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