

# The effect of IL-1 $\alpha$ and nifedipine of cell proliferation and DNA synthesis in cultured human gingival fibroblasts

Naoto Sato<sup>§</sup>, Hiroko Matsumoto<sup>§</sup>, Yoshiaki Akimoto<sup>†</sup>  
and Akira Fujii<sup>§</sup>

Departments of <sup>§</sup>Oral Molecular Pharmacology and <sup>†</sup>Oral Surgery,  
Nihon University School of Dentistry at Matsudo, Chiba, Japan

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**Abstract:** The effect of nifedipine and interleukin- $\alpha$  (IL-1 $\alpha$ ) on the cell proliferation and DNA synthesis was studied in human gingival fibroblasts derived from 5 patients who developed gingival overgrowth (nifedipine responders) and 5 patients who did not develop gingival overgrowth (nifedipine non-responders) in response to nifedipine. Epidermal growth factor was used as a positive control. The fibroblasts derived from nifedipine responders tended to have a numerically greater rate of cell proliferation and DNA synthesis (<sup>3</sup>H-thymidine incorporation) than those from nifedipine non-responders in the presence of nifedipine and IL-1 $\alpha$ . Fibroblasts derived from nifedipine responders showed significantly higher cell proliferation rate in the presence of nifedipine and IL-1 $\alpha$ , than nifedipine or IL-1 $\alpha$  alone on both the second and the fourth day of incubation ( $P < 0.05$ ). A combination of IL-1 $\alpha$  and epidermal growth factor also showed significantly greater cell proliferation than IL-1 $\alpha$  alone on the second day ( $P < 0.05$ ). The DNA synthesis rate with a combination of nifedipine and IL-1 $\alpha$  was higher than that for nifedipine alone on the second day ( $P < 0.01$ ), and IL-1 $\alpha$  alone on the fourth day ( $P < 0.05$ ) in gingival fibroblasts originating from nifedipine responders. These results suggest that the interaction between nifedipine and gingival inflammation might play an important role in the pathogenesis of nifedipine-induced gingival overgrowth. (J. Oral Sci. 47, 105-110, 2005)

**Keywords:** IL-1 $\alpha$ ; nifedipine; gingival overgrowth; gingival fibroblasts; epidermal growth factor.

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## Introduction

Nifedipine is being increasingly used in the treatment of angina pectoris and hypertension and is the most frequently cited causal agent in calcium channel blocker-induced gingival overgrowth (1). Fibroblasts derived from individuals with an enhanced response to nifedipine (nifedipine responders) exhibit a greater rate of cell proliferation and DNA synthesis (<sup>3</sup>H-thymidine incorporation) than those from nifedipine non-responders in the presence of 1  $\mu$ mol/l of calcium channel blockers or phenytoin (2). Excellent reviews on drug-induced gingival overgrowth are available (1-4).

Interleukin-1 (IL-1; IL-1 $\alpha$ , IL-1 $\beta$ ) has been reported to be a potential regulator of fibroblast proliferation (5-9), with significantly higher levels found in gingival crevicular fluid of patients with periodontal disease (10-17). Using the Balb/3T3 fibroblast cell line, Kimball et al. (7) reported that an additive response in terms of cell proliferation when epidermal growth factor (EGF) is used with IL-1 $\alpha$ , and also that IL-1 $\alpha$  is able to induce equivalent levels of proliferation to IL-1 $\beta$  at lower concentrations, but is more potent than IL-1 $\beta$  at a higher concentration. Tomura (18) reported that the growth of human gingival fibroblasts was maximal in a medium containing 100 pg/ml IL-1 $\alpha$  and 500 pg/ml IL-1 $\beta$ . The uronic acid production by gingival fibroblasts is also enhanced in the presence of IL-1, with IL-1 $\alpha$  having a greater effect than IL-1 $\beta$  (18).

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

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Correspondence to Dr. Akira Fujii, Department of Oral Molecular Pharmacology, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho-Nishi, Matsudo, Chiba 271-8587, Japan

Tel: +81-47-360-9344

Fax: +81-47-360-9348

E-mail: afujii@mascat.nihon-u.ac.jp

might be important for gingival overgrowth. IL-1 $\alpha$  predominates (16) in gingival crevicular fluid and a greater amount of nifedipine is found in gingival crevicular fluid in the patients with periodontal disease (19,20). The aim of this study was thus to investigate the simultaneous effect of nifedipine and IL-1 $\alpha$  on cell proliferation and DNA synthesis of gingival fibroblasts from nifedipine responders and non-responders.

## Materials and Methods

### Cells

Gingival fibroblasts were obtained as described previously (2,21). Briefly, cultures of fibroblast-like cells were established from non-inflamed gingival specimens of 5 patients who had developed gingival overgrowth (nifedipine responders) and 5 patients who had not developed gingival overgrowth (nifedipine non-responders) as a result of nifedipine medication (2,21). All the specimens were obtained during gingivectomy, clearance of remaining teeth, or orthopedic surgery of the alveolar ridge from the patient. Each individual gave written informed consent for use of their specimens and the protocol was approved by the Committee on Studies Involving Human Beings of Nihon University School of Dentistry at Matsudo. Disappearance or decreased severity of the overgrowth after discontinuance of nifedipine medication was regarded as indicating a responsive state. Isolation and culture of gingival fibroblasts were performed using methods described by Mod er et al. (22) and Kantor and Hassell (23) with a slight modification (2,21). The cells were incubated under an atmosphere of 5% CO<sub>2</sub> - 95% air at 37°C in a medium of DMEM, supplemented with 10% fetal calf serum (FCS), streptomycin 100 µg/ml, penicillin G 200 U/ml, and amphotericin B 0.2 µg/ml (DMEM-10). When they had reached a confluent phase, the cells were harvested using 0.25% trypsin and 0.02% EDTA in Dulbecco's phosphate-buffered saline (DPBS), washed with DMEM-10, and then re-plated. Homogeneity of fibroblasts was determined by flow cytometry (FACS Vantage, Becton Dickinson, Tokyo, Japan). The fibroblasts proliferated in the logarithmic phase between the 5th and the 8th passage, and cells gave a constant proliferation rate in the presence of nifedipine or phenytoin during the 5th to the 8th passage.

### Cell proliferation assay

The cell proliferation assay was carried out using the method of Kantor and Hassell (23) with a slight modification (2,21). Fibroblasts (approximately  $3 \times 10^4$  cells) in 500 µl of DMEM-10 were allowed to settle in a 24-well plate (Falcon) for 24 h. Cells were washed with DPBS, and the

medium was replaced with DMEM containing 1% FCS and the same antimicrobial agents as above (DMEM-1) for 24 h. Cells were again washed and a further 500 µl of DMEM-10 was added to each well. At this time, 20 µl of 25 µmol/l nifedipine (Sigma Chemical, MO, U.S.A.; final concentration of 1 µmol/l in 0.025% dimethylsulfoxide, solution prepared under a sodium vapor lamp and kept in the dark at 4°C), 20 µl of 2.5 ng/ml IL-1 $\alpha$  (recombinant human IL-1 $\alpha$ , Genzyme, MA, U.S.A.; final concentration of 100 pg/ml in 0.025% dimethylsulfoxide), or 20 µl of 25 ng/ml EGF (Boehringer Mannheim, Tokyo, Japan; final concentration of 1 ng/ml in Hank's solution) was also added. After a 48 or 96 h incubation period, cells were harvested using 0.25% trypsin and 0.02% EDTA in DPBS, and counted using a Coulter Counter ZM (Coulter Electronics, Luton, England). Cells in the absence of drugs but in the presence of 0.025% dimethylsulfoxide served as the control. Data were expressed as a mean of five fibroblastic cell strains obtained from 5 consecutive analysis of each strain. Statistical analysis was performed by the one-way ANOVA at the 95% confidence level.

### DNA synthesis

Fibroblasts (approximately  $6 \times 10^3$  cells) in 100 µl of DMEM-10 were allowed to settle in a 96-well plate (Falcon) for 24 h. The adherent cells were washed and the medium was replaced with DMEM-1. Subsequently, 4 µl of 25 µmol/l nifedipine (final concentration of 1 µmol/l), 4 µl of 2.5 ng/ml of IL-1 $\alpha$  (final concentration of 100 pg/ml), or 4 µl of 25 ng/ml of EGF (final concentration of 1 ng/ml) was added as described above and incubated for 48 h. The medium was then replaced with DMEM-1 and nifedipine, IL-1 $\alpha$ , and EGF were added to make the same final concentrations as above. [<sup>3</sup>H]-thymidine (22.2 KBq, Amersham Life Science, Japan) was added to each well 24 h after the last medium change (48 or 96 h incubation period) and then incorporated into the cell for 24 h. After treatment using 0.25% trypsin and 0.02% EDTA in DPBS, cells were harvested with a cell collector (Micromate 196, Packard Japan, Japan), and the resulting radioactivities on the disc were measured using a direct beta counter (Matrix 96, Packard Japan, Japan). Radioactivity of the cells in the absence of drugs (0.025% dimethylsulfoxide that did not alter the [<sup>3</sup>H]-thymidine incorporation) served as a control. Data were expressed as a mean of 5 strains obtained from 3 consecutive analysis of each strain. Statistical analysis was performed by the one-way ANOVA at the 95% confidence level.

## Results

### Homogeneity and similarity of fibroblasts

Homogeneity of all cell strains used in the present experiment was assessed using flow cytometry. There was no specific difference between cells from nifedipine responders and non-responders in terms of cell size (scatter forward, SCF), and characteristics of the cell surface (FCS) or cell substance (scatter side, SSC). The cell proliferation rate in the presence of nifedipine or phenytoin did not change from the 3rd to 8th passage.

### Cell proliferation

The simultaneous effect of nifedipine and IL-1 $\alpha$  on cell proliferation rate is summarized in Figs. 1a (2nd day) and 1b (4th day). Although cells from nifedipine responders tended to have higher proliferation rates, there was no statistically significant difference between cells from nifedipine responders and non-responders. On both the 2nd and 4th day, the cell proliferation rate of fibroblasts originating from nifedipine responders was significantly higher in the presence of both nifedipine and IL-1 $\alpha$  than nifedipine or IL-1 $\alpha$  alone ( $P < 0.05$ ). A combination of IL-1 $\alpha$  and EGF also resulted in a significantly higher cell proliferation rate than IL-1 $\alpha$  alone on the 2nd day ( $P < 0.05$ ). A combination of IL-1 $\alpha$  and nifedipine resulted in the highest cell proliferation rate of all groups tested on both the 2nd and 4th day.

### DNA synthesis

The simultaneous effect of nifedipine and IL-1 $\alpha$  on DNA synthesis rate, expressed as  $^3\text{H}$ -thymidine incorporation rate, is summarized in Figs. 2a (2nd day) and 2b (4th day). In fibroblasts originating from nifedipine responders, a combination of IL-1 $\alpha$  and nifedipine resulted in a greater DNA synthesis rate than nifedipine alone on the 2nd day ( $P < 0.01$ , Fig. 2a), and IL-1 $\alpha$  alone on the 4th day ( $P < 0.05$ , Fig. 2b). A combination of IL-1 $\alpha$  and EGF also resulted in a higher DNA synthesis rate than IL-1 $\alpha$  alone on the 4th day ( $P < 0.05$ , Fig. 2b). In case of gingival fibroblasts originated from nifedipine non-responders, the same trend was evident. That is, a combination of IL-1 $\alpha$  and nifedipine resulted in a greater DNA synthesis rate than nifedipine alone on the 2nd and 4th day ( $P < 0.001$ , Fig. 2a;  $P < 0.05$ , Fig. 2b, respectively) and IL-1 $\alpha$  alone on the 2nd day ( $P < 0.10$ , Fig. 2a). A combination of IL-1 $\alpha$  and EGF also resulted in a higher DNA synthesis rate than IL-1 $\alpha$  alone on the 4th day ( $P < 0.05$ , Fig. 2b).

## Discussion

In the present study, a combination of nifedipine and IL-1 $\alpha$  enhanced cell proliferation and DNA synthesis of

gingival fibroblasts, particularly in the case of those fibroblasts derived from nifedipine responders. This finding suggests that inflammatory mediators, such as IL-1, might play an important role in gingival overgrowth induced by

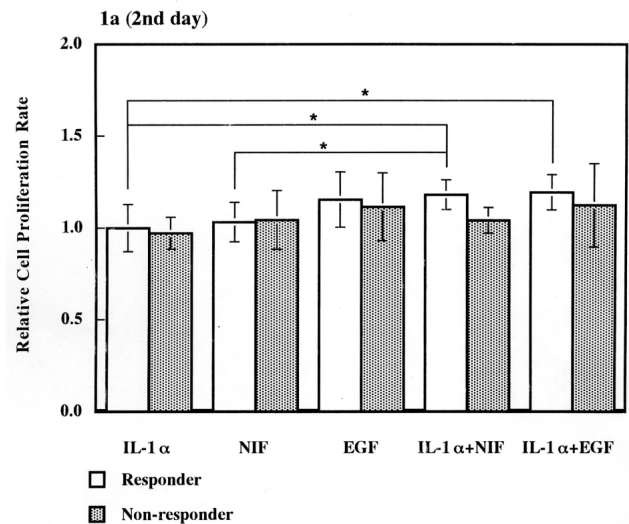


Fig. 1a Effect of nifedipine and IL-1 $\alpha$  on proliferation of fibroblasts originating from nifedipine responders and non-responders, after 48 hours incubation. Values are the mean of five fibroblastic cell strains, with 5 measures of cell proliferation rate for each strain, and are presented as the ratio to the non-drug-treated control. \*  $P < 0.05$ .

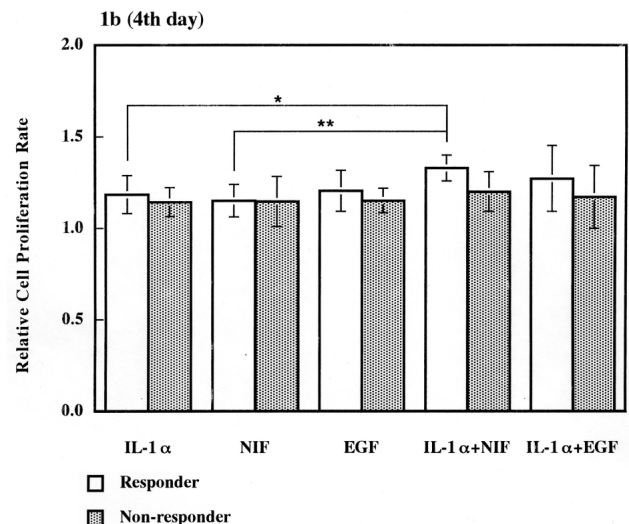


Fig. 1b Effect of nifedipine and IL-1 $\alpha$  on cell proliferation of fibroblasts originating from nifedipine responders and non-responders, after 96 hours incubation. Values are the mean of five fibroblastic cell strains, with 5 measures of cell proliferation rate for each strain, and are presented as the ratio to the non-drug-treated control. NIF, 1  $\mu\text{mol/l}$  nifedipine; EGF, 1 ng/ml; epidermal growth factor; IL-1 $\alpha$ , 100 pg/ml IL-1 $\alpha$ . \*  $P < 0.05$ , \*\*  $P < 0.01$ .

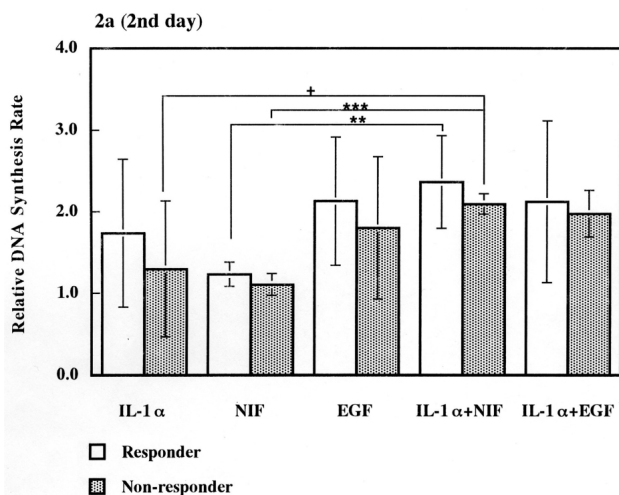


Fig. 2a Effect of nifedipine and IL-1 $\alpha$  on DNA synthesis of fibroblasts originating from nifedipine responders and non-responders, after 48 hours incubation. NIF, 1  $\mu$ mol/l nifedipine; EGF, 1 ng/ml epidermal growth factor; IL-1 $\alpha$ , 100 pg/ml IL-1 $\alpha$ . +  $P < 0.10$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

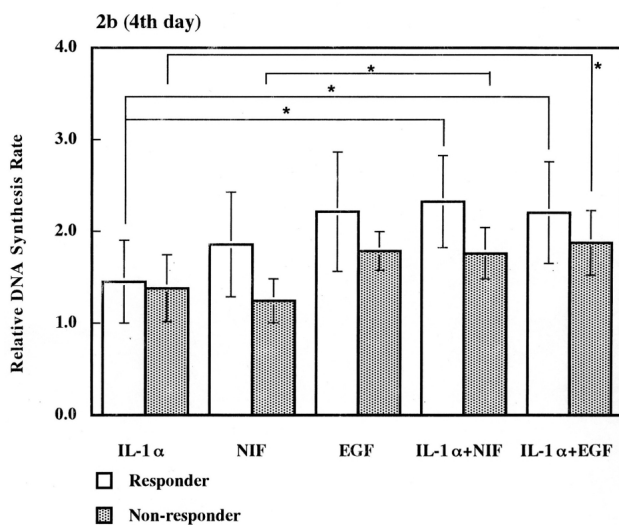


Fig. 2b Effect of nifedipine and IL-1 $\alpha$  on DNA synthesis of fibroblasts originating from nifedipine responders and non-responders, after 96 hours incubation. NIF, 1  $\mu$ mol/l nifedipine; EGF, 1 ng/ml epidermal growth factor; IL-1 $\alpha$ , 100 pg/ml interleukin-1 $\alpha$ . \*  $P < 0.05$ .

nifedipine.

IL-1 $\alpha$  plays a central role in the gingival inflammatory process. It has been found at significantly higher levels in gingival crevicular fluid of patients with periodontal disease (10-16), and Fujihara (25) reported that the production of both IL-1 $\alpha$  and IL-1 $\beta$  were induced in human peripheral monocytes stimulated with endotoxin from periodontopathic bacteria. Several studies have also shown that IL-

1 is a potent stimulator of fibroblast proliferation (5,7-9,26,27), and Inokuchi et al. (28) demonstrated that IL-1 $\alpha$  stimulates cell proliferation through Ca<sup>2+</sup>/calmodulin-dependent pathway in NIM 1 cells, a human thyroid cell line established from a patient with thyroid papillary adenocarcinoma. The findings of recent papers that the IL-1 blockers, CK-119 and CK-122 (29) and 1-methyl hydrazine analogs (30,31) inhibit the cell proliferation in human lung fibroblasts and fibroblast-like cells, respectively, also suggest that IL-1 enhances cell growth in fibroblasts.

Nifedipine is found in significantly higher levels in gingival crevicular fluid in nifedipine induced-gingival lesions (18,19), and we have previously demonstrated that nifedipine stimulates cell proliferation in human gingival fibroblasts, particularly those from nifedipine responders (2).

The presence of both nifedipine and IL-1 $\alpha$  in the gingival tissues is thus likely in patients with gingival inflammation and receiving nifedipine, and, based on the present findings, their simultaneous presence could result in enhanced cell proliferation and DNA synthesis with resultant gingival overgrowth.

Chemical mediators, such as bradykinin, substance P and histamine have been demonstrated to enhance the effect of IL-1 $\alpha$ . Kimball et al. (6) reported that bradykinin, at concentrations between 10<sup>-8</sup> and 10<sup>-5</sup> mol/l, moderately promoted [<sup>3</sup>H]-TdR incorporation in vitro in the BALB/3T3 cells, and that substance P at approximately 3  $\times$  10<sup>-9</sup> to 3  $\times$  10<sup>-7</sup> mol/l demonstrated minor proliferative activity.

Table 1 Source of cell strains

Patient	Age	Sex	Nifedipine Dose mg/day	Site Duration yrs	Degree of gingival overgrowth <sup>a</sup>
Nifedipine responder					
1	66	F	20	1.0 6,	buccal; gingiva 3
2	65	F	20	4.0 3,	labial; gingiva 3
3	74	M	20	2.0 6,	buccal; gingiva 2
4	55	M	30	3.0 1 1,	labial; gingiva 2
5	67	M	15	8.0 1 1,	labial; gingival 2
Nifedipine non-responder					
6	66	F	40	2.0 521 1,	buccal; alveolar ridge none
7	70	F	20	2.0 45,	buccal; gingiva none
8	51	M	20	7.0 6,	buccal; alveolar ridge none
9	69	M	10	7.0 3 3,	buccal; gingival none
10	74	F	20	4.0 345,	buccal; gingiva none

<sup>a</sup>Degree of gingival overgrowth: 2, moderate; 3, severe; none, clinically healthy with a Gingival Index of 0, and a Bleeding Index of 0.

However, when the cells were cultured with IL-1 plus substance P or IL-1 plus bradykinin, the ensuing proliferative responses were consistently magnified 2-fold above the anticipated additive response caused by IL-1 in combination with either of these neuropeptides. These chemical mediators might also enhance the effect of nifedipine

One limitation of this study is that gingival samples were taken from different anatomical sites. However, our previous findings using an animal model (24) demonstrated that growth of gingival fibroblasts in the presence of nifedipine or phenytoin did not differ by site.

In conclusion, the results of the present study suggest that the presence of IL-1 $\alpha$  results in greater cell proliferation and DNA synthesis than in the presence of nifedipine alone. While this effect was evident for both nifedipine responder and non-responder gingival fibroblasts, responder fibroblasts showed a greater response. These findings suggest that the interaction between nifedipine and gingival inflammation might play an important role in the pathogenesis of nifedipine-induced gingival overgrowth.

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