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Research Paper

The effect of Nifedipine on the expression of type I collagen in gingival fibroblasts ☆

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Abstract

Objective: To investigate the effect of nifedipine(calciumchannel blocker) on the expression of collagen in gingival fibroblasts invitro. **Methods:**Primarily gingival fibroblasts were cultured and incubated with various concentrations of nifedipine(108 μg/L, 360 μg/L and 1200 μg/L)for 5 days. Gingival fibroblasts were primarily cultured derived from nifedipine responders and non-responders in the presence of 360 μg/L nifedipine. Enzyme-linked immunosorbent assay was used to evaluate the amount of type I collagen. Cell proliferation was measured by cell counting with evaluating MTT value. **Results:**The expressions of collagen and cell proliferation were significantly different among the high concentration groups and the others on the fifth day, especially higher in 360 μg/L and 1200 μg/L groups and also different among nifedipine responders and non-responders. **Conclusion:**The expression of collagen and cell proliferation may be concerned with the biological mechanism for gingival overgrowth.

Key words: fibroblast; gingival hyperplasia; type I collagen; nifedipine

INTRODUCTION

Nifedipine, the dihydropyridines calcium-channel blocking agent, is used widely to treat symptoms of coronary heart disease(CHD) and hypertension. Gingival overgrowth may occur as a side-effect in patients receiving nifedipine medication. However, the molecular mechanism of drug-induced gingival overgrowth is not definitively known. Many studies showed that collagen (COL), one of the connec tivetissue extracellular matrix, (ECM) especially typeⅠcollagen(COL-1), accumulates in hyperplasic tissue induced by nifedipine. Under physiological conditions, for COL-1(one of the major components in the gingival connective tissue) a dynamic balance between its synthesis and degradation, is essential. The objective of this study is to investigate the effect of nifedipine on the expression of collagen in gingival fibroblasts, suggesting a biological mechanism for gingival overgrowth *in vitro*.

MATERIALS AND METHODS

Cells culture

Human normal gingival fibroblasts were obtained during surgical removal of impacted wisdom teeth from a healthy volunteer. The specimens of nifedipine responders and non-responders were obtained from patients who had developed gingival overgrowth as a result of nifedipine medication and patients who did not develop gingival overgrowth following nifedipine medication. The gingiva was cultured by the tissue explants method.

Drugs

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To prepare the nifedipine(Nanjing NO.1 Pharmacy Co., Ltd) medium concentrations of 108 μg/L, 360 μg/L and 1200 μg/L, containing 0.01% pure ethanol(vehicle, Sigma, USA) and ascorbic acid(50 μg/ml, Sigma, USA) which is essential for collagen synthesis was used.

Cell proliferation

Normal human gingival fibroblasts were cultured with nifedipine medium as follows: Group Ⅰ:108 μg/L of nifedipine; Group Ⅱ:360 μg/L of nifedipine; Group Ⅲ: 1200 μg /L of nifedipine; Group Ⅳ: only medium as control. Fibroblasts were harvested for cell counting after 1, 3 and 5 days. Four gingival fibroblast strains(GF-1, -2,-3,-4) from nifedipine responders and non-responders were cultured in DMEM, supplemented with 360 μg/L nifedipine. We observed the effect of cell proliferation by evaluating MTT value after 1, 3 and 5 days.(GF-1,-2: nifedipine non-responders;GF-3,-4:nifedipine responders)

Enzyme-linked immunosorbent assay(ELISA)

The production of type Ⅰ collagen was assayed using a special ELISA. The supernatant of media was collected after 1, 3 and 5 days. The protein contents of COL-1 were measured by a human COL-1 ELISA kit (Zhangshan Goldenbridge Biotechnology Co., Ltd) according to the manufacturer's instructions.

Statistical analysis

The data was expressed as mean ± SD and was analyzed by ANOVA with SPSS software. Significant differences were noted at a *P*-value of < 0.05.

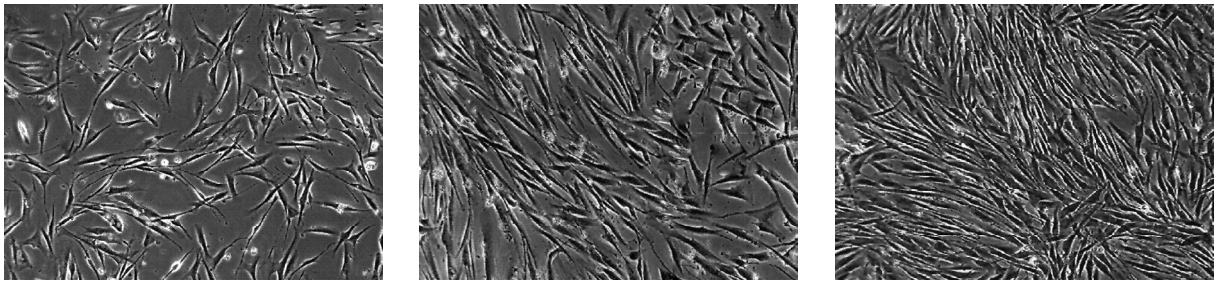
RESULTS

Cell growth

Generally, the number of cells of every group increased from 1 to 5 days. ANOVA application demonstrated in 3 days between each group multiplies were not obvious different, but in the 5th day each group displayed significant difference(***Tab 1, Tab 2*** and ***Fig 1***). The results showed statistical difference between nifedipine responders and non-responders on days 1, 3 and 5(***Tab 3*** and ***Tab 4***).

ELISA

Fig 2 illustrated that the expression of COL-1 coincided with the results of cell proliferation. In the 1st and 3rd day between each group synthesis were not obvious different, but on the 5th day each group displayed significant difference. The results showed statistical difference between nifedipine responders and non-responders on 1, 3 and 5 days(***Fig 3***).



108 μg/L group 360 μg/L group 1200 μg/L group

Fig 1 Microphotographs of cultured gingival fibroblasts on 5th day(× 100)

Tab 1 **Cell proliferation in 4 groups** ($\times 10^4, \bar{x} \pm s$)

group Ⅰ	group Ⅱ	group Ⅲ	group Ⅳ	*F*	*P*	Value
0 th day	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00		
1 st day	2.88 ± 0.18	5.00 ± 2.83	6.50 ± 2.12	2.70 ± 0.141	2.107	0.24
3 rd day	6.25 ± 2.12	11.50 ± 1.41	11.50 ± 2.12	5.55 ± 3.32	3.820	0.11
5 th day	9.00 ± 3.18	18.00 ± 1.41	30.50 ± 4.95	8.00 ± 4.24	15.950	0.01

Tab 2 **Post HOC Comparisons on 4 groups**

	group	Ⅰ	Ⅱ	Ⅲ
5 th day	Ⅱ	0.029		
	Ⅲ	0.004	0.065	
	Ⅳ	0.004	0.049	0.799

DISCUSSION

Nifedipine and dihydropyridines(the calcium blocking agent) is widely used in the treatment of ischemic cardiovascular disease, such as angina pectoris and hypertension[1]. Since the first report[2] of nifedipine-induced gingival overgrowth, many clinical cases[3-4] indicated that gingival overgrowth may occur as a side-effect in patients receiving nifedipine medication. The prevalence rate of nifedipine-induced gingival overgrowth is 20% to 83%[5].

The mechanism of drug-induced fibrosis is not fully understood. In general, it is possible that it is related

Tab 3 **Cell proliferation in 4 groups of nifedipine responders and non-responders** ($OD_{492}, \bar{x} \pm s$)

GF-1	GF-2	GF-3	GF-4	*F*	*P*	valve
1 st day	0.0087 ± 0.0020	0.0073 ± 0.0011	0.0240 ± 0.0015	0.0142 ± 0.0022	18.042	0.000
3 rd day	0.0178 ± 0.0010	0.0120 ± 0.0011	0.0458 ± 0.0030	0.0275 ± 0.0025	48.942	0.000
5 th day	0.0313 ± 0.0024	0.0217 ± 0.0028	0.1318 ± 0.0082	0.0963 ± 0.0082	75.428	0.000

Tab 4 **Post HOC comparisons on 4 groups**

	group	GF-1	GF-2	GF-3
1 st day	GF–2	0.603		
	GF–3	0.000	0.000	
	GF–4	0.041	0.013	0.001
3 rd day	GF–2	0.078		
	GF–3	0.000	0.000	
	GF–4	0.007	0.000	0.000
5 th day	GF–2	0.274		
	GF–3	0.000	0.000	
	GF–4	0.000	0.000	0.001

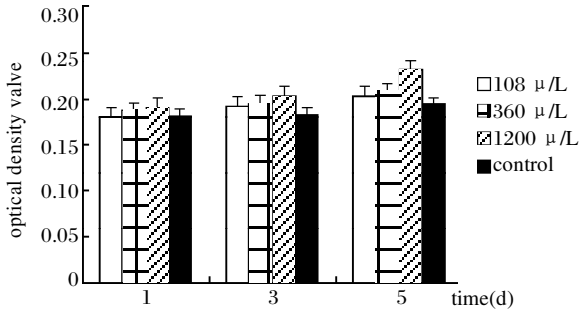


Fig 2 Effect of nifedipine on the expression of type Ⅰ collagen of normal gingival fibroblasts

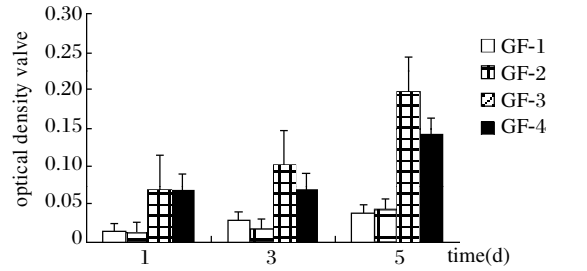


Fig 3 Effect of nifedipine on the expression of type Ⅰ collagen of nifedipine responders and non-responders

with cell proliferation of gingival fibroblasts promoted by nifedipine. Fujii[6-9] et al. demonstrated that fibroblasts from patients reacting to nifedipine gave trends toward better cell proliferation rate, DNA synthesis as compared with those without nifedipine. However this data didn't agree with the results of Salo[10], who showed that 100 μg/L and 200 μg/L nifedipine had no effect on the cell proliferation in normal human gingival fibroblasts. Ellis[11] et al reported that the concentration of nifedipine in gingival crevicular fluid in patients receiving nifedipine was 0.92～9.30 μg/ml,15～90 fold higher than that in plasma. In this study, we chose various concentrations of nifedipine according to concentration gradient:108 μg/L、360 μg/L and 1200 μg/L. Our results showed that cell proliferation is dependent on time and dose, it is that long time and high concentration promoted cell growth, which agree with the results of our early studies.

Many studies do not support that nifedipine can result in cell proliferation, but suggest that collagen accumulation may work. For COL-1, one of the components in the gingival connective tissue and extracellular matrix, a dynamic balance between its synthesis and degradation is essential. Spolidorio[12] *et al* demonstrated a disturbance tissue homeostasis leaded to an accumulation of extracellular matrix in gingival overgrowth, in particular COL-1. Nakao[13] *et al* reported that in hyperplastic gingiva compared to normal tissues, the level of expression of collagen increased. Kataoka[14] *et al* reported that the contents of collagen in rats gingival connective tissues (which were fed by nifedipine) lead to gingival overgrowth compared with the normal rats, and was shown as obviously increased by the results of immunohistochemistry. All those suggested that extracellular matrix degradation depressed and collagen deposited by nifedipine may be the mechanism of gingival overgrowth. We showed that the results were the same as cell proliferation, depending on time and dose. Our data suggested that collagen secreted by fibroblasts was raised by nifedipine, which proved collagen stacked in histology.

In our study, the results of cell proliferation and ELISA showed that the high concentration of nifedipine could stimulate the expression of type Ⅰ collagen. But for the molecular mechanism, such as the gene regulation of gingival fibroblast extracellular matrix by nifedipine is still to be investigated.

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Current approaches of genome-wide association studies

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With rapid advances in high-throughput genotyping technology and the great increase in information available on SNPs throughout the genome, genome-wide association(GWA) studies have now become feasible. By testing associations of diseases or traits with a large number of SNPs that capture most of the genetic information across the genome, GWA approaches can identify variants that directly or indirectly confer risk. The systematic and objective nature of GWA increases the likelihood of identifying risk variants and may reveal novel mechanisms. The power of this approach has been empirically demonstrated by the recent success of GWA studies in many diseases,including cancers.

Approaches to design, implement, and analyze GWA studies will evolve rapidly. I will first discuss study design issues in terms of study populations, sample sizes, SNP platforms, and multiple stages of confirmation. I will then discuss quality control issues in genotyping and data analyses. I will describe several approaches to analyze GWA data, including genetic models, imputation, and gene-environment and gene-gene interactions. Finally, I will discuss the utility of results from GWA studies.