J Periodont Res 2006; 41; 316–321

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2006.00864.x

Synergism between nifedipine and cyclosporine A on the incorporation of [^{35}S]sulfate into human gingival fibroblast cultures *in vitro*

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Flynn JC, Henderson JS, Johnson RB. Synergism between nifedipine and cyclosporine A on the incorporation of [^{35}S]sulfate into human gingival fibroblast cultures in vitro. *J Periodont Res 2006; 41; 316–321. © Blackwell Munksgaard 2006*

Background and Objective: We assessed the effects of cyclosporine A and nifedipine on the *in vitro* incorporation of [^{35}S]sulfate into gingival fibroblast cell cultures derived from responder and nonresponder subjects who had received an organ transplant followed by a therapeutic regimen using a combination of those drugs.

Material and Methods: Gingival fibroblasts were isolated from responder and nonresponder subjects and maintained *in vitro*. Prior to cell harvest, gingival interleukin-1β concentrations were determined by enzyme-linked immunosorbent assay (ELISA). Cells were untreated or exposed to either 10^{-7}–10^{-10} M nifedipine or 100–500 ng/ml cyclosporine A. Incorporation of [^{3}H]proline or [^{35}S]sulfate into the cell cultures was determined by liquid scintillation analysis. In addition, the effects of 400 ng/ml cyclosporine A + 10^{-7} M nifedipine and 400 ng/ml cyclosporine A + 10^{-10} M nifedipine on incorporation of [^{35}S]sulfate into the cell cultures was determined. Data were compared by factorial analysis of variance (ANOVA) and a posthoc Tukey's test.

Results: Gingiva from responders contained significantly more interleukin-1β than gingiva from nonresponders ($p < 0.01$). The cell cultures derived from responders incorporated significantly more [^{35}S]sulfate than those derived from nonresponders following exposure to either cyclosporine A or 10^{-7} M nifedipine. In addition, the exposure of fibroblasts derived from gingival overgrowth to either 400 ng/ml cyclosporine A + 10^{-7} M nifedipine or 400 ng/ml cyclosporine A + 10^{-10} M nifedipine significantly increased or decreased, respectively, the incorporation of [^{35}S]sulfate into the cultures.

Conclusion: The therapeutic combination of cyclosporine A and nifedipine could be a significant risk factor for gingival overgrowth in subjects susceptible to either agent. The mechanism for overgrowth could include edema secondary to increased sulfated-glycosaminoglycan (sGAG) synthesis by fibroblasts, but further investigation is required.

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Key words: cyclosporine A; gingival fibroblasts; gingival overgrowth; nifedipine

Accepted for publication November 21, 2005

Gingival overgrowth is a disfiguring condition that affects 15–20% of individuals receiving nifedipine (NIF) (1) and 25–81% of those receiving cyclosporine A therapies (2). Cyclosporine A is commonly utilized as an immunosuppressive drug to prevent the rejection of transplanted organs (3). In addition, Cyclosporine A is often used in the treatment of various autoimmune diseases, including diabetes mellitus (4), Behcet's disease (5), and systemic lupus erythematosis (6). A common side-effect of cyclosporine A therapy is hypertension, which is often controlled with vasodilator medications, such as NIF (7), which is, itself, a risk factor for gingival overgrowth. Thus, cyclosporine A and NIF are often administered together, especially in organ transplant patients.

Numerous studies suggest that subject-specific risk factors are important in the development of gingival overgrowth (8–10). When exposed to either cyclosporine A or NIF, gingival overgrowth becomes evident in many subjects (responders); however, others do not experience gingival overgrowth (nonresponders) (11). When cyclosporine A and NIF are administered together, gingival overgrowth often becomes even more severe in responders (7). There is no general agreement concerning possible physiological differences between responders and nonresponders, although distinct subpopulations of gingival fibroblasts, macrophages, and lymphocytes have been identified that probably contribute to the individual susceptibility for gingival overgrowth in responders (9,12).

Fibroblasts increase the synthesis of collagens, proteoglycans, and other extracellular matrix components within the gingiva of responders receiving cyclosporine A or NIF (13–16). Several studies have been carried out into the effects of cyclosporine A on the composition of human gingival sulfated-glycosaminoglycan (sGAG) (17–22), but no studies have been reported of the effects of NIF on gingival sGAG concentration. In addition, there are conflicting data regarding the specific effects of cyclosporine A on gingival sGAG synthesis and accumulation. For instance, there is evidence for either elevated (17–19,21) or no change (18,20) in the tissue concentrations of sGAG within gingival overgrowth tissues from subjects receiving cyclosporine A.

Although case histories of renal transplant patients describe enhanced gingival overgrowth in patients receiving both cyclosporine A and NIF (23,24), there are no studies of the specific synergistic mechanisms underlying the adverse effects of these agents. In addition, there are no comparative studies of the differences in rates and patterns of sGAG synthesis by gingival fibroblasts obtained from responder and nonresponder organ transplant patients. Because of the widespread use of cyclosporine A and NIF, study of their combined effects on the gingival extracellular matrix would be useful information for the design of both preventive and therapeutic procedures for gingival overgrowth. Thus, it seemed worthwhile to test the individual and combined effects of cyclosporine A and NIF on sGAG accumulation of *in vitro* cultures of fibroblasts derived from these patients.

Materials and methods

Isolation of fibroblasts

Human gingiva biopsies were obtained from renal transplant patients receiving cyclosporine A + NIF therapy. Eight subjects had evidence of gingival overgrowth (responders) and eight subjects had no evidence of gingival overgrowth (nonresponders). All subjects were patients at the dental outpatient clinic of the University of Mississippi Medical Center. The Institutional Review Board of the University of Mississippi Medical Center approved the project and the informed consent document.

All subjects were Caucasian males receiving the following medications: prednisone (10–15 mg every other day), NIF (20–30 mg three times/d), cyclosporine A (310–330 mg/d), and azanthropine (25–50 mg/d). gingival overgrowth was removed from responders by gingivectomy. Gingival papillae were obtained from nonresponders prior to routine extraction of the adjacent teeth. Each tissue sample was minced with a scalpel and the cells were removed by digestion in 0.1% (w/v) collagenase (*Clostridium histolyticum*, Type 1; Sigma Chemical Company, St Louis, MO, USA). The cells were maintained in α-minimal essential medium containing 10% (v/v) heat-inactivated fetal bovine serum and 1% (v/v) antibiotics (Sigma Chemical Company). When the cells became confluent, they were detached from the substrate by treatment with trypsin–EDTA (Sigma Chemical Company) and plated into tissue culture dishes. The cells were studied during passages 3–6.

Determination of the interleukin-1β concentration

Some tissue fragments from each biopsy were completely solubilized by grinding in phosphate-buffered saline (PBS). Before grinding, the tissue was blotted, weighed in a microbalance, and then placed in a volume of PBS sufficient to ensure a dilution of 10 mg of tissue per ml of PBS. A standard bicinchoinic acid assay (Pierce Chemical Company, Rockford, IL, USA) was used to assess the protein concentration of each tissue aliquot. The absorbance of each well was read in a microplate spectrophotometer at 570 nm, and protein concentrations were calculated from a standard curve. To determine the level of interleukin-1β, tissue aliquots were assayed by enzyme-linked immunosorbent assay (ELISA), using a commercial kit (Quantikine®; R & D Systems, Minneapolis, MN, USA). The absorbance of each well was read in a microplate spectrophotometer at 450 nm and the concentration of interleukin-1β was calculated from a standard curve. Each tissue sample was assayed in duplicate. Data were expressed as pg of interleukin-1β/mg of protein.

Exposure to test media

A total of 10^5 cells were seeded in 60-mm dishes and maintained for 6 d in α-minimal essential medium containing

10% (v/v) heat-inactivated fetal bovine serum, 1% (v/v) antibiotics, 50 μg/ml ascorbic acid, and 0 (control) or 10^{-7}–10^{-10} M NIF, 100–500 ng/ml cyclosporine A, or a combination of the target serum dosage for cyclosporine A (400 ng/ml) and the NIF concentrations with maximal (400 ng/ml cyclosporine A + 10^{-7} M NIF) or minimal (400 ng/ml cyclosporine A + 10^{-10} M NIF) effects on incorporation of [^{35}S]sulfate into the cell culture. On day 6, the cells were exposed to serum-free medium containing either 1 μCi/ml [^{3}H]proline (L-2, 3-[^{3}H]proline; specific activity, 57 Ci/mmol) or 1 μCi/ml [^{35}S]sulfate (25–40 Ci/mg of sulfur; Amersham Life Sciences, Arlington Heights, IL, USA). On day 7, the cell monolayer was detached by treatment with trypsin–EDTA and an aliquot was removed in order to carry out a quantitative assessment of cell number using a Coulter counter. All experiments were repeated four times.

Each cell culture was solubilized and then dialyzed for 24 h against 50 mM Tris/HCl buffer (pH 7.6), containing 5 mM $CaCl_2$. An aliquot of the dialysate from each cell culture, exposed to either [^{35}S]sulfate or [^{3}H]proline, was dissolved in scintillation fluid and the radioactivity determined by liquid scintillation analysis.

Statistical analysis

Mean cell counts, decay per min/10^6 cells, and gingival interleukin-1β concentrations (pg/mg protein) were determined for each sample and group, and mean values were compared by factorial analysis of variance and a posthoc Tukey's test using SPSS 12.0.2™ for Windows. Mean values were considered to be significantly different when $p < 0.05$.

Results

Interleukin-1β gingival concentrations

The mean interleukin-1β concentration within solubilized gingival overgrowth samples from responders was significantly greater (12.64 ± 1.81 pg/mg of protein) than within the normal gingiva (NG) samples from nonresponders (4.89 ± 0.72 pg/mg of protein) ($p < 0.01$).

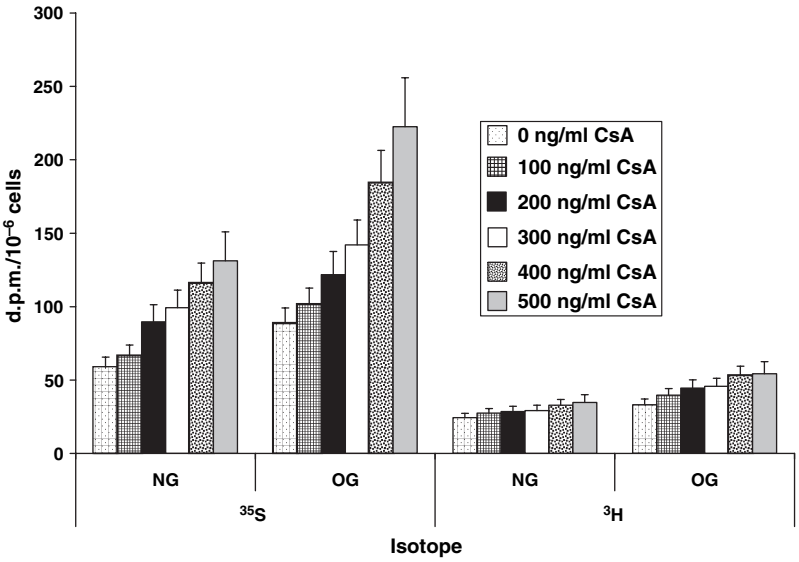

Fig. 1. Incorporation of [^{35}S]sulfate or [^{3}H]proline into fibroblast cultures derived from either normal or overgrowth gingiva exposed to various concentrations of cyclosporine A. $n = 8$ for each group. CsA, cyclosporine A; d.p.m., decay per minute; NG, normal gingiva; OG, overgrowth gingiva.

Exposure of fibroblasts to cyclosporine A

Exposure of fibroblasts obtained from both NG and gingival overgrowth to 300–500 ng/ml cyclosporine A significantly increased the incorporation of [^{35}S]sulfate into the cell cultures, compared with the control (300 ng/ml cyclosporine A, $p < 0.05$; 400–500 ng/ml cyclosporine A, $p < 0.001$) (Fig. 1). In addition, the incorporation of [^{35}S]sulfate into the fibroblast cell cultures obtained from gingival overgrowth was significantly greater than that incorporated into cultures obtained from NG following exposure to 100–500 ng/ml cyclosporine A (100–300 ng/ml cyclosporine A, $p < 0.05$; 400–500 ng/ml cyclosporine A, $p < 0.001$) (Fig. 1). Incorporation of [^{3}H]proline into the fibroblast cell cultures obtained from gingival overgrowth was significantly greater than incorporation by cultures obtained from NG following exposure to 200–500 ng/ml cyclosporine A ($p < 0.001$). Moreover, the incorporation of [^{3}H]proline into cultures exposed to 300–500 ng/ml cyclosporine A was significantly greater than that of the control ($p < 0.001$) (Fig. 1).

Exposure of fibroblasts to NIF

In general, the cell cultures obtained from gingival overgrowth incorporated significantly more [^{35}S]sulfate than those obtained from NG, regardless of the NIF concentration (10^{-7} M and 10^{-8} M NIF, $p < 0.001$; 10^{-9} M and 10^{-10} M, $p < 0.05$) (Fig. 2). Administration of 10^{-7} to 10^{-9} M NIF resulted in the incorporation of a significantly greater concentration of [^{35}S]sulfate by the cell cultures obtained from NG ($p < 0.05$) compared with the control (Fig. 2). In addition, 10^{-7} M to 10^{-10} M NIF significantly increased the incorporation of [^{35}S]sulfate into the cell cultures compared with the control (10^{-7} M and 10^{-8} M NIF, $p < 0.001$; 10^{-9} M and 10^{-10} M NIF, $p < 0.05$). Exposure to 10^{-7} to 10^{-9} M NIF produced no significant differences in the incorporation of [^{3}H]proline into the fibroblast cell cultures obtained from either NG or gingival overgrowth. However, exposure to 10^{-10} M NIF significantly reduced the incorporation of [^{3}H]proline into the cultures

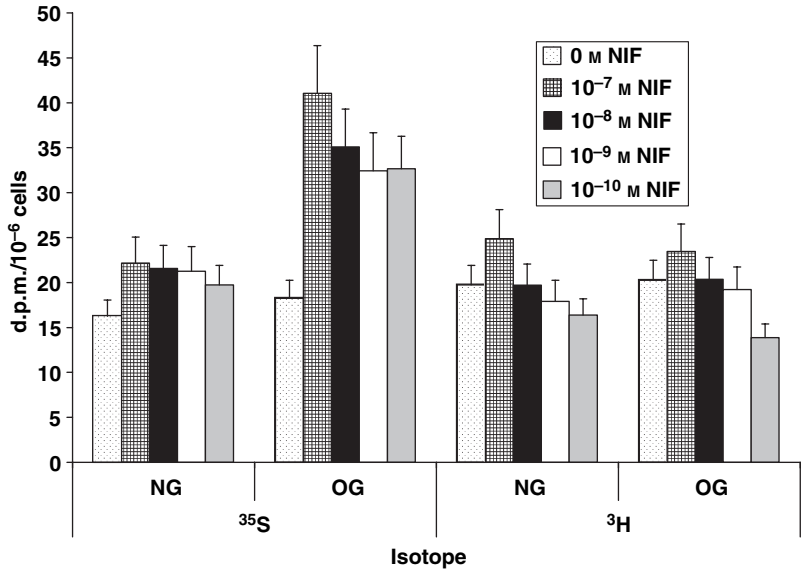

Fig. 2. Incorporation of [^{35}S]sulfate or [^{3}H]proline into fibroblast cultures derived from either normal or overgrowth gingiva exposed to various concentrations of nifedipine. $n = 8$ for each group. d.p.m., decay per minute; NG, normal gingiva; OG, overgrowth gingiva.

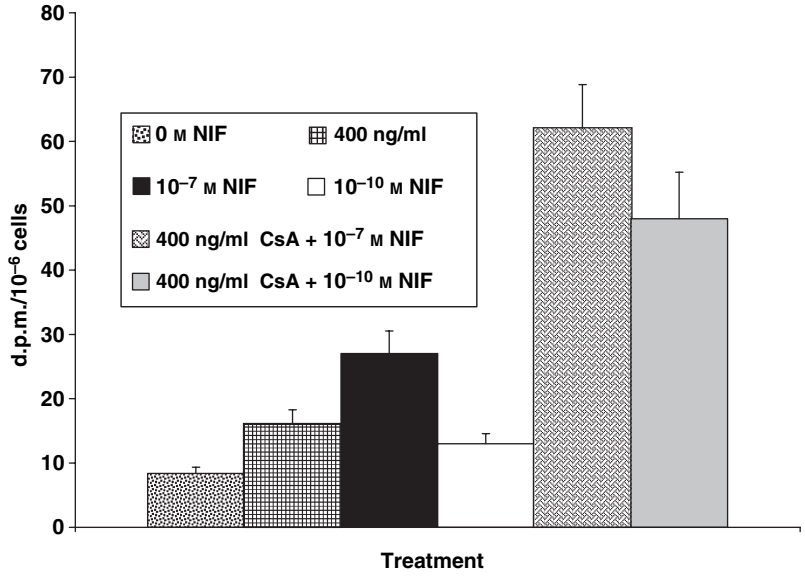

Fig. 3. Incorporation of [^{35}S]sulfate into fibroblast cell cultures derived from overgrowth gingiva exposed to either 400 ng/ml cyclosporine A, 10^{-7} M or 10^{-10} M nifedipine (NIF), or combinations of 400 ng/ml cyclosporine A + 10^{-7} M or 10^{-10} M NIF. $n = 8$ for each group. CsA, cyclosporine A; d.p.m., decay per minute.

obtained from gingival overgrowth ($p < 0.001$) (Fig. 2).

Synergism between cyclosporine A and NIF

When combined, 10^{-7} M NIF + 400 ng/ml cyclosporine A and 10^{-10} M NIF + 400 ng/ml cyclosporine A significantly increased the incorporation of [^{35}S]sulfate into fibroblast cell cultures obtained from gingival overgrowth, compared with the control, or administration of 400 ng/ml cyclosporine A or 10^{-7} M NIF alone (Fig. 3, Table 1). In contrast, 10^{-10} M NIF + 400 ng/ml cyclosporine A significantly decreased the incorporation of [^{35}S]sulfate into those cultures (Fig. 3, Table 1).

Discussion

Our study reports that cyclosporine A, NIF, and cyclosporine A + NIF significantly increase the net [^{35}S]sulfate accumulation within cell cultures of fibroblasts obtained from gingival overgrowth, as compared with those from NG and controls. There were numerous differences in matrix synthesis by fibroblasts obtained from gingival overgrowth and NG when exposed to NIF and cyclosporine A, supporting data from other studies (10). To our knowledge, this is the first report of synergistic enhancement of sGAG accumulation within fibroblast cell cultures obtained from gingival overgrowth following exposure to cyclosporine A and NIF, providing additional information concerning the biological mechanisms for gingival overgrowth following exposure to these agents. The factors for this enhancement included the type of drug, drug concentration, and source of the cells [responder (OG) vs. nonresponder (NG)]. Although all gingival tissue donors had received similar drug therapy, not all had sites of gingival overgrowth, confirming previous reports of drug-sensitive subpopulations of gingival fibroblasts within gingiva (9,11).

Our data were derived from assays of incorporation of commonly used radioisotopes for the assessment of collagenous protein and sGAG accumulation within the extracellular matrix. [^{3}H]Proline is incorporated both into collagenous proteins and into proteoglycan cores (25,26). [^{35}S]Sulfate is predominately incorporated into sGAG (27), but small amounts may also be incorporated into glycoprotein (21,28).

There has been considerable controversy concerning the effects of NIF on gingival fibroblast metabolism. In addition, there is some controversy about the effect of NIF on cyclosporine A-induced gingival overgrowth, with studies suggesting no difference (18), enhancement (29), or reduced (30) accumulation of gingival extracellular

Table 1. Significant differences between the mean incorporation of [^{35}S]sulfate into fibroblast cultures derived from overgrowth gingiva exposed to 400 ng/ml cyclosporine A (CsA), 10^{-7} M or 10^{-10} M nifedipine (NIF), or combinations of 400 ng/ml cyclosporine A + 10^{-7} M or 10^{-10} M NIF (n = 8 for each group)

	Treatment					
Treatment	0 (Control)	400 ng/ml CsA	10^{-7} M NIF	10^{-10} M NIF	400 ng/ml CsA + 10^{-7} M NIF	400 ng/ml CsA + 10^{-10} M NIF
0 (Control)	***	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.001$	$p < 0.001$
400 ng/ml CsA	$p < 0.001$	***	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
10^{-7} M NIF	$p < 0.001$	$p < 0.001$	***	$p < 0.001$	$p < 0.001$	$p < 0.001$
10^{-10} M NIF	$p < 0.05$	$p < 0.05$	$p < 0.05$	***	$p < 0.001$	$p < 0.001$
400 ng/ml CsA + 10^{-7} M NIF	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	***	$p < 0.001$
400 ng/ml CsA + 10^{-10} M NIF	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	***

matrix, compared with NG. Exposure to NIF has been reported to increase the rate of collagen synthesis (16) and the incidence of gingival fibrosis (15) by fibroblasts obtained from both gingival overgrowth and NG (31). Our study confirms those data by reporting enhanced collagenous protein accumulation within fibroblast cell cultures obtained from both NG and gingival overgrowth following exposure to NIF *in vitro*. In contrast, previous studies have reported that NIF exposure produces either no effect (32), or decreased accumulation of collagen within the cell culture matrix (10,33), which is not supported by our data.

There are conflicting data concerning the concentrations of sGAG within gingival overgrowth, with some reporting higher concentrations (17,19,21,27), and others reporting no difference (18,20), from NG. Our data demonstrate a dose-dependent increase in sGAG accumulation within the fibroblast cell cultures obtained from both NG and gingival overgrowth following exposure to NIF, which is a novel observation.

There is also considerable controversy concerning the effects of cyclosporine A on gingival fibroblast metabolism. Our data confirm that exposure to cyclosporine A increased net collagenous protein deposition into the cell cultures (34), the effect being greater with gingival overgrowth than with NG. We extended previous studies by reporting that a dose-dependent *in vitro* exposure of either NG or gingival overgrowth fibroblasts to cyclosporine A significantly increased [^{35}S]sulfate incorporation of the cell cultures, with the effect being greater in gingival overgrowth than in NG (18–21,35).

A small number of human studies have reported interactions between NIF and cyclosporine A regarding deposition of the gingival extracellular matrix components (23,24). A histomorphometric study reported enhanced extracellular matrix collagen density in rats following exposure to both cyclosporine A and NIF (36). Our data demonstrate significant synergistic effects of cyclosporine A and NIF on sGAG accumulation within the fibroblast cell cultures obtained from gingival overgrowth, extending previous studies and providing a possible biological mechanism for gingival overgrowth in patients receiving both medications. As both collagenous protein and sGAG deposition into the cell cultures was enhanced by cyclosporine A + NIF *in vitro*, the gingiva might enlarge in responders as a result of fibrosis and edema, resulting from the ability of sGAG to bind water *in vivo*.

Our data also suggest that fibroblasts could have been transformed within gingival overgrowth *in vivo*, possibly as a result of the exposure to higher levels of pro-inflammatory cytokines in that microenvironment. Previous studies have reported alteration of the immune response in gingival overgrowth (12). In addition, a previous study reported that gingival overgrowth had significantly higher interleukin-1β concentrations than NG (31). Both interleukin-1β and TNF-α have been reported to increase the accumulation of collagen protein within the extracellular matrix following NIF exposure *in vitro*, suggesting that elevated concentrations of pro-inflammatory cytokines within the gingiva could be important risk factors for gingival overgrowth *in vivo* (37,38). This phenomenon could result from enhancement of fibroblast metabolism by pro-inflammatory cytokines within their environment. A previous study reported that periodontal treatments that reduced gingival inflammation also reduced the severity of gingival overgrowth (39,40) by lowering the concentrations of extracellular sGAG (27).

Thus, enhanced net sGAG and collagenous protein accumulation within fibroblast cell cultures obtained from gingival overgrowth and exposed to cyclosporine A + NIF *in vitro*, suggests that this drug combination could be a significant risk factor for repeated episodes of gingival overgrowth in organ transplant patients *in vivo*, which extends previous results (23,24). In addition, our *in vitro* data suggest that the removal of either cyclosporine A or NIF from therapy could reduce the net incorporation of sGAG into the extracellular matrix in gingival overgrowth (responder) patients *in vivo* and, as a result, may reduce the incidence of gingival overgrowth. Additional human clinical trials are required to confirm this preliminary hypothesis.

References

1. Barclay S, Thomason JM, Idle JR, Seymour RA. The incidence and severity of nifedipine-induced gingival overgrowth. *J Clin Periodontol* 1992;**19:**311–314.
2. Seymour RA, Jacobs DJ. Cyclosporin and the gingival tissues. *J Clin Periodontol* 1992;**19:**1–11.
3. Calne RY, Rolles K, White DJ *et al*. Cyclosporin A in clinical organ grafting. *Transplant Proc* 1981;**13:**349–358.
4. Stiller CR, Dupre J, Gent M *et al*. Effects of cyclosporine immunosuppression in insulin-dependent diabetes mellitus of recent onset. *Science* 1984;**223:**1362–1367.
5. Nussenblatt RB, Palestine AG, Rook AH, Wacker WB, Seher I, Gery I. Treatment of intraocular inflammatory disease with cyclosporin-A. *Lancet* 1983;**2:**235–238.
6. Isenberg D, Snaith M, Al-Klader A *et al*. Cyclosporin relieves arthralgia, causes angioedema. *N Engl J Med* 1980;**303:**754.
7. Thomason JM, Seymour RA, Rice N. The prevalence and severity of cyclosporin and nifedipine induced gingival overgrowth. *J Clin Periodontol* 1993;**20:**37–40.
8. Bartold PM. Regulation of human gingival fibroblast growth and synthetic activity by cyclosporine-A *in vitro*. *J Periodont Res* 1989;**24:**314–321.
9. Hassell TM, Romberg E, Sobhani S, Leskol L, Douglas R. Lymphocyte-mediated effects of cyclosporine on human fibroblasts. *Transplant Proc* 1988;**20:**993–1002.
10. McKevitt KM, Irwin CR. Phenotypic differences in growth, matrix synthesis and response to nifedipine between fibroblasts derived from clinically healthy and overgrown gingival tissue. *J Oral Pathol Med* 1995;**24:**66–71.
11. Hassell TM, Hefti AF. Drug-induced gingival overgrowth: old problem, new problem. *Crit Rev Oral Biol Med* 1991;**2:**103–137.
12. Pernu HE, Knuuttila ML, Huttenen KR, Tilikainen ASK. Drug-induced gingival overgrowth and class II major histocompatibility antigens. *Transplantation* 1994;**57:**1811–1813.
13. Narayanan AS, Bartold PM. Biochemistry of periodontal connective tissues and their regeneration: a current perspective. *Connect Tissue Res* 1996;**34:**191–201.
14. Seymour RA, Thomason JM, Ellis JS. The pathogenesis of drug-induced gingival overgrowth. *J Clin Periodontol* 1996;**23:**165–175.
15. Tipton DA, Fry HR, Dabbous MK. Altered collagen metabolism in nifedipine-induced gingival overgrowth. *J Periodont Res* 1995;**29:**401–409.
16. Fujii A, Matsumoto H, Nakao S, Teshigawara H, Akimoto Y. Effect of calcium-channel blockers on cell proliferation, DNA synthesis and collagen synthesis of cultured gingival fibroblasts derived from human nifedipine responders and non-responders. *Arch Oral Biol* 1994;**39:**99–104.
17. Mariani G, Calastrini C, Carinci F, Bergamini L, Calastrini F, Stabellini G. Ultrastructural and histochemical features of the ground substance in cyclosporin A-induced gingival overgrowth. *J Periodontol* 1996;**67:**21–27.
18. Martins RC, Werneck CC, Rocha LA, Feres-Filho EJ, Silva LCF. Molecular size distribution analysis of human gingival glycosaminoglycans in cyclosporin- and nifedipine-induced overgrowths. *J Periodont Res* 2003;**38:**182–189.
19. Newell J, Irwin CR. Comparative effects of cyclosporin on glycosaminoglycan synthesis by gingival fibroblasts. *J Periodontol* 1997;**68:**443–447.
20. Rocha LAG, Martins RCL, Werneck CC, Feres-Filho EJ, Silva LCF. Human gingival glycosaminoglycans in cyclosporine-induced overgrowth. *J Periodont Res* 2000;**35:**158–164.
21. Zebrowski EJ, Pylypas SP, Odlum O *et al*. Comparative metabolism of ^{3}H-glucosamine by fibroblast populations exposed to cyclosporine. *J Periodontol* 1994;**65:**565–567.
22. Wondimu B, Reinholt FP, Modeer T. Stereologic study of cyclosporin A-induced gingival overgrowth in renal transplant patients. *Eur J Oral Sci* 1995;**103:**199–206.
23. Radwan-Oczko M, Boratynska M, Klinger M, Zietak M. Risk factors of gingival overgrowth in kidney transplant recipients treated with cyclosporine A. *Ann Transplant* 2003;**8:**57–62.
24. O'Valle F, Mesa F, Aneiros J *et al*. Gingival overgrowth induced by nifedipine and cyclosporin A. Clinical and morphometric study with image analysis. *J Clin Periodontol* 1995;**22:**591–597.
25. Sodek J. A new approach to assessing collagen turnover by using a microassay. A highly efficient and rapid turnover of collagen in rat periodontal tissues. *Biochem J* 1976;**160:**243–246.
26. Sodek J. A comparison of the rates of synthesis and turnover of collagen and non-collagen proteins in adult rat periodontal tissues and skin using a microassay. *Arch Oral Biol* 1977;**22:**655–665.
27. Vardar S, Baylas H, Zihnioglu F, Budundeli N, Emingil G, Attila G. Gingival tissue proteoglycan and chondroitin-4-sulphate levels in cyclosporin A-induced gingival overgrowth and the effects of initial periodontal treatment. *J Clin Periodontol* 2005;**32:**634–639.
28. Young RW. The role of the Golgi complex in sulfate metabolism. *J Cell Biol* 1973;**57:**175–189.
29. Morisaki I, Fukui N, Fujimori Y, Murakami J, Dakoku H, Amano A. Effects of combined oral treatments with cyclosporine A and nifedipine or diltiazem on drug-induced gingival overgrowth in rats. *J Periodontol* 2000;**71:**438–443.
30. Chiu H-C, Fu E, Chiang C-Y, Liu D. Does nifedipine aggravate cyclosporin-induced gingival overgrowth? An experiment in rats. *J Periodontol* 2001;**72:**532–537.
31. Henderson JS, Flynn JC, Tucci MA, Tsao AK, Zebrowski EJ. Site-specific variations in metabolism by human fibroblasts exposed to nifedipine *in vitro*. *J Oral Pathol Med* 1997;**26:**6–10.
32. Nishikawa S, Tada H, Hamasaki A *et al*. Nifedipine-induced gingival hyperplasia: a clinical and *in vitro* study. *J Periodontol* 1991;**62:**30–35.
33. Salo T, Oikarinen KS, Oikarinen AI. Effect of phenytoin and nifedipine on collagen gene expression in human gingival fibroblasts. *J Oral Pathol Med* 1990;**19:**404–407.
34. Schincaglia GP, Forniti F, Cavallini R, Piva R, Calura G, del Senno L. Cyclosporin-A increases type I procollagen production and mRNA level in human gingival fibroblasts *in vitro*. *J Oral Pathol Med* 1992;**21:**181–185.
35. Mariani G, Calastrini C, Carinci F, Marzola R, Calura G. Ultrastructural features of cyclosporine A-induced gingival hyperplasia. *J Periodontol* 1993;**64:**1092–1097.
36. Spoildorio LC, Spoildorio DM, Neves KA *et al*. Morphological evaluation of combined effects of cyclosporin and nifedipine on gingival overgrowth in rats. *J Periodont Res* 2002;**37:**192–195.
37. Johnson RB. Synergistic enhancement of collagenous protein synthesis by human gingival fibroblasts exposed to nifedipine and TNF-alpha *in vitro*. *J Oral Pathol Med* 2002;**32:**408–413.
38. Johnson RB, Zebrowski EJ, Dai X. Synergistic enhancement of collagenous protein synthesis by human gingival fibroblasts exposed to nifedipine and interleukin-1-beta *in vitro*. *J Oral Pathol Med* 2000;**29:**8–12.
39. Bullon P, Machuca G, Martinez-Sahuquillo A *et al*. Evaluation of gingival and periodontal conditions following causal periodontal treatment in patients treated with nifedipine and diltiazem. *J Clin Periodontol* 1996;**23:**649–657.
40. Somacarrera ML, Lucas M, Scully C, Barrios C. Effectiveness of periodontal treatments on cyclosporine-induced gingival overgrowth in transplant patients. *Br Dent J* 1997;**183:**89–94.