Treatment of Cyclosporine-Induced Gingival Overgrowth with Azithromycin-Containing Toothpaste

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Objectives: **Gingival overgrowth is a complication of cyclosporine therapy following organ transplantation. Oral azithromycin is frequently used to treat this complication. This study examined the efficacy of local azithromycin, in the form of toothpaste, against cyclosporine-induced gingival overgrowth.**

Materials and Methods: **Twenty stable renal transplanted patients (10 men and 10 women) with gingival hyperplasia were randomly assigned to a test group and a control group. Azithromycin-containing toothpaste had 85 mg azithromycin per gram of toothpaste. Both toothpastes were prescribed b.i.d., each time using 1.5 cm, for 1 month. All participants received scaling, root planing, polishing, and oral hygiene instructions, at least 4 weeks prior to initiation of the study. Gingival overgrowth index, bleeding on probing, blood urea nitrogen, creatinine, and serum cyclosporine levels were measured at baseline, and then again in the second and fourth weeks after tooth brushing. Patient satisfaction with the toothpastes was evaluated by a visual analogue scale. The stability of clinical responses was followed for 3 months after cessation of the toothpastes.**

Results: **Gingival overgrowth index decreased significantly in the azithromycin-containing toothpaste group (from 1.1 ± 0.56 to 0.51 ± 0.47, *P* < .001); however, in the control group, this decrease was not significant (*P* = .22). Bleeding on probing also decreased significantly in patients in the azithromycin-containing toothpaste group compared with controls (*P* = .001). When compared with baseline levels, trough levels of cyclosporine, blood urea nitrogen, and creatinine did not change in either of the groups. Patients in the control group were more satisfied with the toothpaste than were patients in the test group (53 vs 38).**

Conclusions: **Azithromycin-containing toothpaste is an effective, simple, and noninvasive treatment for cyclosporine-induced gingival overgrowth.**

Key words: *Azithromycin, Gingival overgrowth, Cyclosporine*

Gingival overgrowth (GO) is an adverse effect of immunosuppressive therapy with cyclosporine (CsA) treatment following renal transplantation. Its prevalence ranges from 21% to 35% [1]. The variability of clinical expression of CsA-related GO implies a multifactorial pathogenesis. CsA blood concentration, plaque/gingivitis level, bacterial lipopolysaccharides, and alteration of calcium ion cellular influx have been suggested as possible factors [2, 3]. The first documented incidence of CsA-induced GO was published in 1983 by Wysocki and coworkers [4]. GO may interfere with normal oral functions, leave patients with an unpleasant appearance, carry psychological impacts, influence compliance with medical therapy, and make it difficult to maintain optimal oral hygiene [5]. The latter, in turn, may exacerbate GO via bacterial overgrowth [2]. The immunosuppressive actions of CsA may allow tissue invasion by micro-organisms, which causes a secondary inflammatory response [6, 7].

Azithromycin already has been shown to have in vitro activity against a wide range of pathogens [8, 9] and to be more effective than other macrolide antibiotics against many common pathogens [10]. Azithromycin therapy for 3 to 5 days previously has been shown to improve gingival hyperplasia [11-13].

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Acknowledgments: *This work was supported by a grant from Drug Applied Research Center (DARC), Tabriz University of Medical Sciences, Tabriz, Iran*

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Experimental and Clinical Transplantation (2006) 1: 420-424

Azithromycin concentrates in fibroblasts and phagocytes [14] and is transported to areas of inflammation as a result of its chemotactic effects on the phagocytes [15]. On the other hand, tissue and intracellular concentrations of azithromycin have been found up to 100 [15] to 200 [16] times their normal concentration in the serum [17].

Therefore, it is theoretically logical that if systemic azithromycin could be replaced by a local form, such as toothpaste—and if its anti-GO effect could be clarified—that tooth brushing with this compound would be a much simpler and safer method of treating CsA-induced GO.

In this study, we tested the efficacy and applicability of azithromycin-containing toothpaste for CsA-induced GO. To our knowledge, this is the first study of its kind published in the English literature.

Materials and Methods

Twenty patients (10 women, 10 men; mean age, 36.80 ± 12.89 years) with CsA-induced GO were enrolled. All of the subjects were informed regarding the purpose of the study and the products being evaluated before written informed consent was obtained. All of the patients had received their graft at least 12 months earlier, between 1 to 7 years. At the time, they were under conventional triple immunosuppressive therapy composed of CsA, prednisone, and either azathioprine or mycophenolate mofetil. Inclusion criteria were stable renal function (serum creatinine < 2 mg/dL) without episodes of acute rejection during the past 3 months; GO involving at least the anterior teeth; and negative drug history for phenytoin, calcium channel blockers, or macrolide consumption during the prior 6 months. All of the participants were instructed in effective oral hygiene measures for dental plaque removal, and they were allowed to enter the study only if their plaque indices were less than 20%. Patients also had undergone scaling, root planning, and professional cleaning of the tooth surfaces to decrease the inflammatory component of the gingival enlargements, at least 4 weeks prior to the beginning of the study.

Ten patients (5 men, 5 women; mean age, 39.20 ± 12.90 years) in the intervention group received azithromycin-containing toothpaste (ACTP), which had been formulated in the Department of Pharmaceutics, Faculty of Pharmacy, Tabriz Medical Science University of Iran. The toothpaste consisted of sodium lauryl sulfate as a foaming agent, calcium dehydrates as abrasive agents, sodium saccharine and sodium menthol as flavors, sodium carboxy-methyl cellulose as a thickening agent, propylene glycol as a humectant, vitamin C as the buffer to avoid the irritating effect of high pH and azithromycin (85 mg/g). The control group (5 men, 5 women) used placebo toothpaste, which had the same ingredients except for the azithromycin. In both groups, a 50-gram tube of toothpaste was delivered, and patients were instructed to use 1.5 cm of it, as a usual dentifrice, 2 times per day, for 4 weeks. Plaque index, probing depth, gingival overgrowth index (GOI), and bleeding on probing (BOP) were measured at baseline and at the second and fourth weeks after the intervention. At each time interval, intraoral digital photographs were taken from anterior and bilateral posterior views and were presented to an independent expert clinician to verify the GOI. According to GO scoring system [18], each examined site (gingival portion) may obtain a score from 0 (meaning no gingival enlargement) to 3 (meaning severe enlargement). The patient's overall GOI was considered by calculating the mean GOI of all the examined sites. Probing depth was recorded on the midbuccal and distal aspect of all teeth present and was measured to the nearest 1 mm using a William's probe. BOP was based on the classification by Löe & Silness as previously described by Newman and coworkers [18].

GOI was assessed again after 3 months (ie, 2 months after cessation of toothpastes), using the same method, to detect clinical response evolution over time. The patients' opinions of the taste, smell, and appearance of the placebo and the test toothpastes also were assessed using a visual analogue scale. These parameters were analyzed by paired and unpaired *t* and Wilcoxon signed rank tests. Values for *P* less than .05 were considered significant.

Results

Patients' characteristics (age, sex, date of renal transplantation, dosages of CsA, serum CsA level, BOP, plaque index, probing depth, and serum creatinine levels) were relatively similar in both groups (Table 1). Only GOI was moderately higher in the experimental group (0.56 ± 0.51 vs 1.1 ± 0.56, $P = .039$).

All of the participants satisfactorily completed the study. They returned their emptied toothpaste tubes on the 18th day of the study (so they could receive more toothpaste) and also at the end of study. No significant differences were found between the residual volume of

Table 1. Characteristics of the patients in the 2 groups

Patient characteristics	Controls	Cases	*P*
Male to female ratio	5/5	5/5	----
Age (years)	34 ± 13	39 ± 13	.42
Date of transplantation (years)	7.6 ± 4.4	5.3 ± 4	.25
Cyclosporine dose (mg/day)	187.5 ± 27	175 ± 71.5	.61
Serum cyclosporine (mic/mL)	213.5 ± 130	260.5 ± 109	.39
Serum creatinine (mg/dL)	1.3 ± 0.8	1.5 ± 0.9	.21
BOP	0.35 ± 0.18	0.44 ± 0.13	.26
Probing depth (mm)	7.9 ± 1.3	8.8 ± 1.9	.22
Plaque index	44.3 ± 16.8	54.1 ± 15.3	.19
GOI	0.57 ± 0.51	1.1 ± 0.56	.039

BOP, bleeding on probing; GOI, gingival overgrowth index

Table 2. Gingival parameters of subjects using 2 different toothpastes. Data are shown at the baseline (before) and at the end of 1 month (after).

Gingival Parameters	Cases		Controls	
	Before	After	Before	After
Plaque index	54.1 ± 15.3	17.9 ± 6.2	44.3 ± 16.9	15.3 ± 4
	P = .005		*P* = .005	
Bleeding on probing	0.44 ± 0.14	0.1 ± 0.08	0.35 ± 0.18	0.18 ± 0.14
	P = .005		*P* = .005	
Probing depth	8.9 ± 1.9	7.4 ± 1.2	7.9 ± 1.3	7.35 ± 1.3
	P = .005		*P* = .038	
Gingival overgrowth index	1.1 ± 0.56	0.51 ± 0.47	57 ± 0.51	0.52 ± 0.44
	P = .005		*P* = .22	

the ACTP and placebo dentifrices, which demonstrated acceptable compliance of the patients. There were no subjective or objective adverse effects, systemically or locally, whether using the ACTP or placebo dentifrice.

In patients using ACTP, all of the gingival parameters improved significantly (Table 2). The improvements for plaque index, BOP, probing depth, and GOI were 36.2, 0.34, 1.5, and 0.59, respectively ($P = .005$). Interestingly, with regard to the first 3 parameters, improvement also was seen in the control patients (ie, plaque index, BOP, and probing depth, which were decreased up to 29 [$P = .005$], 0.17 [$P = .005$], and 0.55 [$P = .039$], respectively). The difference in GOI between the groups was not significant (0.57 ± 0.51 vs 0.52 ± 0.44, $P = .22$).

Regardless of the toothpaste used, serum CsA trough levels did not change during the study period (260.6 ± 109.3 μL and 213.5 ± 130.0 μL before vs 260.8 ± 179.3 μL and 214.6 ± 84.8 μL) after application of either ACTP and placebo toothpaste, respectively. Also, we did not find any correlation between serum CsA levels and severity of gingival overgrowth. Longevity of renal transplantation, CsA dosages, age, and sex did not impact gingival overgrowth.

The GOI remission effect of topical azithromycin

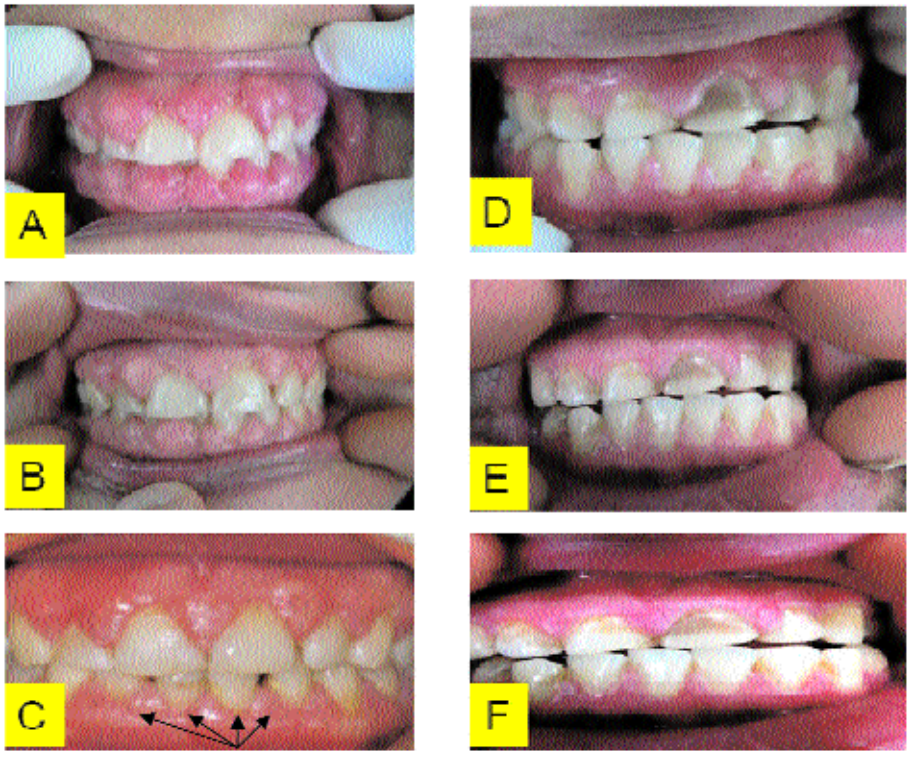

Figure 1. Gingival overgrowth before and after treatment with toothpastes. Figure 1A demonstrates gingiva at the baseline. Figures 1B and C show the same gingiva after using of azithromycin-containing toothpaste at the end of first (B) and third month (C), respectively. Figures 1D, E, and F show another patient who used placebo toothpaste at the baseline, after 1 month, and after 3 months, respectively. Bases of teeth appear only in those teeth treated with the azithromycin-containing toothpaste (arrows).

was still obvious even after 3 months of cessation of using of the toothpaste at follow-up appointments (Figure 1).

Although all patients were compliant and used the toothpastes regularly as prescribed by the physician, satisfaction was greater in patients in the control group. A visual analogue scale, which assessed patient satisfaction of the toothpastes, based on smell and taste, showed 53 in the placebo group were satisfied while 38 in the ACTP group were. We assume that this was due to the bitter taste of the suspended azithromycin in the ACTP toothpaste. During the 6-month study, toothpaste stability was measured periodically with Fourier Transform Infrared, which demonstrated stable area-under-the-curve concentrations for the functional groups of azithromycin in the ACTP.

Discussion

Although many drugs, such as CsA, can directly induce gingival enlargement, secondary inflammatory processes due to dental plaque accumulation and other local factors, may frequently intensify the effects of drugs on gingival tissues [3]. Indeed, the majority of gingival enlargements have a combined etiology.

Without the removal of infected plaques, effective treatment of gingival disorders will fail [19]. Therefore, we attempted to treat the inflammatory components of gingival disease before initiating the study by removing gingival inflammation, clinically, and thus eliminating this as a confounding factor.

Several oral antibiotics, such as metronidazole and azithromycin, have been studied in the treatment of CsA-induced GO with varying amounts of success [20]. Patient compliance and adverse effects (ie, diarrhea, nausea, abdominal pain, and vomiting) of drugs could be problems with oral azithromycin therapy [21]. Azithromycin is a macrolide antibiotic closely related to erythromycin A. It is an interesting drug with a similar spectrum of activity to erythromycin but has enhanced potency against gram-negative organisms. It is concentrated in fibroblasts and phagocytes [14] and is transported to areas of inflammation as a result of chemotactic effects exerted on the phagocytes [16] thus targeting the drug at those sites. Local concentrations of azithromycin in gingival tissue greatly exceed the concomitant serum levels by 200 times [16]. Based on these data, we tried to improve the CsA-induced GO locally, with toothpastes, as a convenient and complication-free mode of therapy.

In a study by Nafar and coworkers [22], the authors observed that azithromycin therapy, systemically or locally, has no significantly beneficial effect on GO over professional dental cleaning and dental care. However, their study was not associated with well-controlled subjects, was not blindly performed, and their results were not conclusive.

Maizumi and coworkers [23] found that topical application of azithromycin to the gingival crevice may require a slow-release, long-life matrix, not only to decrease the adverse effects of these macrolides, but also to increase their clinical efficacy. These authors concluded that a topical azithromycin MIC90 concentration (the concentration required to inhibit the growth of 90% of the bacteria) has little effect on the growth and differentiation of the periodontal ligament. However, their data could not be extrapolated to in vivo situations, because gingival gene expression would be prevented by topical application of azithromycin at a tissue concentration of 2.0 μg/mL (as even this is sufficient to inhibit microbial growth).

All of our 20 patients, irrespective of their being in the control group or the ACTP group, had good oral hygiene during the treatment. Although GOI was moderately higher in patients in the ACTP group at the beginning of the study (simply by chance), it does not suggest that oral hygiene could be of increased utility and efficacy in more severe cases, as all of the patients reached an acceptable oral hygiene level at the beginning of the study, which seemed to be enough for all the effects of dental plaque on gingival condition to subside.

Regular visits (biweekly) of the patients in the both groups demonstrated that the gingival enlargement did not recur in the ACTP group, and clinical symptoms improved at 3 months follow-up (Figure 1). This implies that topical azithromycin therapy has lasting therapeutic effects on gingival tissue, which are similar to the long-term effects of oral azithromycin therapy, which have been described previously [24].

Many of the questions that arise from our data should be answered as soon as possible with well-designed randomized clinical trials. We must know the exact mechanism(s) of azithromycin's antiproliferative effect. As we did not measure blood concentrations of azithromycin, we cannot be certain that a systemic effect of the drug absorbed by the tongue, or even via unintentional swallowing of little amounts of ACTP, was not responsible for the regression of GO.

Although we found no correlation between CsA trough level and GOI, it is interesting that we did find

a significant correlation between saliva CsA concentration (at CsA level) and GOI (data not shown). The correlation implies that salivary secretions of CsA, but not systemic CsA, produce gingival pathology after organ transplantation.

In summary, we showed for the first time that topical azithromycin has a beneficial effect on CsA-induced gingival disorders. Its use is convenient for patients and has no adverse effects.

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