Pediatr Transplantation 2004: 8: 60–64
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Pediatric Transplantation

Trial of metronidazole vs. azithromycin for treatment of cyclosporine-induced gingival overgrowth

Chand DH, Quattrocchi J, Poe SA, Terezhalmy GT, Strife CF, Cunningham RJ. Trial of metronidazole vs. azithromycin for treatment of cyclosporine-induced gingival overgrowth.
Pediatr Transplantation 2004: 8: 60–64. © 2004 Blackwell Munkagaard

Abstract: Gingival overgrowth usually characterized by increased cellular growth of gingival fibroblasts appears to be multifactorial. In patients receiving CyA for more than 3 months, the incidence can approach 70% and can be attributed to pharmaceutical immunosuppression. Case reports have reported regression of overgrowth with both metronidazole and azithromycin. The goal of this study was to determine the efficacy of metronidazole and azithromycin in reducing CyA-induced gingival overgrowth. Twenty-five patients were included in this double-blinded randomized study. All patients were receiving CyA as medically indicated and diagnosed with gingival overgrowth by a dentist. Patients were randomized to receive either 5-days of azithromycin or 7-days of metronidazole given at baseline only. The extent of gingival overgrowth was measured at 0, 2, 4, 6, 12, and 24 wk. Fourteen patients at CCF and 11 patients at CCHMC were studied. Repeated measures ANOVA was performed to assess differences within and between groups. Gingival overgrowth at baseline was not statistically different between groups. The mean degree of gingival overgrowth after treatment was different across all time intervals ($p = 0.0049$) showing azithromycin to be more effective than metronidazole. Therapy with azithromycin offers an effective alternative to the management of CyA-induced gingival overgrowth.

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Key words: cyclosporine – gingival overgrowth – metronidazole – azithromycin

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Accepted for publication 28 January 2003

Gingival overgrowth secondary to drug therapy (phenytoin, calcium-channel blocking agents, and CyA) has been recognized for decades (1). It can be attributed to increased growth of gingival fibroblasts (2–4). There seems to be an association between poor oral hygiene and the incidence and magnitude of drug-induced gingival overgrowth (5, 6). Although the degree of gingival overgrowth does not appear to correlate to CyA serum concentrations, there may be a threshold concentration of the drug or its metabolites that is necessary for this effect to occur (7, 8). The immunosuppressive actions of CyA may allow tissue invasion by microorganisms, which causes a secondary inflammatory response (8, 9). CyA-induced gingival overgrowth has been reported in up to 70% of patients, usually becomes evident within 3 months after initiation of therapy, and often regresses when the drug is discontinued (1, 7, 8). Although transplant recipients are urged to practice rigorous oral hygiene to minimize gingival effects, such preventive measures are seldom successful and ultimately most patients who develop gingival overgrowth require multiple surgical interventions (gingivectomy) during the course of CyA therapy (8). Anecdotal reports have attributed a salutary effect of both metronidazole and azithromycin on the magnitude and incidence of CyA-induced gingival overgrowth (5, 10–12). However, very few double-blinded randomized trials with statistical power have been conducted to confirm the efficacy of either drug.

Abbreviations: CyA, cyclosporine; CCF, the Cleveland Clinic Foundation; CCHMC, Cincinnati Children's Hospital Medical Center.

Patients and methods

A double-blinded randomized trial was conducted at CCF from March 1998 through January 1999 and at CCHMC from November 2000 to March 2002. Approval was obtained from the Institutional Review Boards and is in accordance with the Helsinki Declaration of 1975. Patients were eligible if they were receiving CyA and were found to have gingival overgrowth as evaluated by a dentist. Baseline measurements were obtained by periodontal probing, which allows for assessment of periodontal tissue structural status by mechanically probing the gingival sulcus region. It is performed by insertion (with gentle pressure) of a periodontal probe (calibrated in mm) between the tooth and gingival and other periodontal tissues and measuring the depth of insertion. In this study, measurements were recorded for the mesial, distal, and buccal/facial gingiva of all existing maxillary and mandibular teeth relative to the cementoenamal junction. As the instrument used to measure gingiva is a calibrated instrument and the technique is standard evaluation procedure, examiner calibration, and inter-examiner agreement were not deemed necessary.

Each patient was randomized to receive either a 5 day course of azithromycin: 10 mg/kg on day 1 (500 mg maximum) followed by a daily dose of 5 mg/kg (maximum 250 mg) for 4 days; or metronidazole 45 mg/kg/day divided into three doses daily for 7 days. Both groups were re-evaluated at 2, 4, 6, 12, and 24 wk post-treatment. A control group was not used, as the natural progression of gingival overgrowth has been well documented (1, 4, 6). At each visit, the patient had gingival photographs taken from frontal and side views to document tissue response to therapy. All participants were blinded to the study medication. Serum CyA levels were monitored at routine nephrology clinic visits and were maintained between 150 and 300 ng/mL. Patients who completed a minimum of four of six visits were included. If a baseline measurement was not available for a tooth, it was excluded.

A *t*-test was used to determine if mean age differed among drug groups. ANOVA using repeated measures was used to determine if the mean degree of gingival overgrowth differed across time intervals after treatment with each drug. Statistical significance was determined using a p-value of ≤ 0.05, except when adjustments were made for multiple comparisons, at which time significance was based on a p-value of ≤ 0.0021. There were 24 comparisons made: comparing each drug across time and then comparing the two drugs to each other at each time interval. For example, for azithromycin, the mean measurement at baseline was compared with the mean measurement at 2 wk, 4 wk, 6 wk, 13 wk, and 26 wk respectively. Analysis was performed using the MIXED procedure in SAS version 8.2®, with an appropriate covariance matrix to account for the unequally spaced time intervals. Based on these statistical methods, a 1-mm change over time was considered statistically significant, with a s.d. of 0.8 mm. A statistical power of 85% and a significance level of 0.05 were assumed. The α-level was set to 0.05 for two-tailed testing.

Results

Twenty-eight patients were enrolled (14 at CCF, and 11 at CCHMC); however, three patients

Table 1. Demographic information

ID	Gender	Ethnicity	Age	Drug	Reason for CyA use
1	Female	White	25	A	Post-transplant
2	Male	White	13	M	Post-transplant
3	Female	White	22	A	Post-transplant
4	Female	White	16	M	Post-transplant
5	Male	White	21	M	Post-transplant
6	Female	White	22	M	Post-transplant
7	Male	White	20	A	Post-transplant
8	Female	Other	14	M	Steroid-resistant NS
9	Male	White	17	A	Post-transplant
10	Male	White	12	M	Post-transplant
11	Male	White	13	A	Post-transplant
12	Male	White	26	M	Post-transplant
13	Female	Black	13	A	Post-transplant
14	Male	White	16	A	Post-transplant
15	Male	White	20	M	Post-transplant
16	Male	Other	15	A	Post-transplant
17	Male	Black	24	M	Post-transplant
18	Female	Black	13	M	Post-transplant
19	Male	White	14	M	Post-transplant
20	Male	White	3	A	Post-transplant
21	Male	White	15	M	Post-transplant
22	Male	White	33	A	Post-transplant
23	Male	White	17	M	Post-transplant
24	Male	Other	12	A	Steroid-resistant NS
25	Female	White	21	M	Post-transplant
	Male: 68% Female: 32%	Caucasian: 76% African-American: 12% Other: 12%	Mean age: 17.48 ± 6.06	azithromycin (A): 44% Metronidazole (M): 56%	

Table 2. Does mean age differ among those on azithromycin vs. metronidazole?

Drug	N	Mean	s.d.	p-value
A	11	17.18	7.82	0.8327*
M	14	17.71	4.55	

*Pooled *t*-test.

Table 3. Mean (actual and adjusted) gingival measurements across time intervals after treatment with azithromycin vs. metronidazole

Drug	Time interval (wk)	Actual mean* ± s.e. (mm)	Least-square mean ± s.e. (mm)
Azithromycin (n = 11)	Baseline	12.87 ± 0.40	12.87 ± 0.31
	2	11.27 ± 0.33	11.27 ± 0.31
	4	12.20 ± 0.36	11.32 ± 0.32
	6	11.37 ± 0.29	11.37 ± 0.31
	12	11.44 ± 0.28	11.26 ± 0.32
	26	11.41 ± 0.34	11.36 ± 0.33
Metronidazole (n = 14)	Baseline	13.13 ± 0.29	13.13 ± 0.27
	2	12.71 ± 0.26	12.27 ± 0.27
	4	12.93 ± 0.30	13.16 ± 0.27
	6	11.68 ± 0.23	12.19 ± 0.27
	12	12.49 ± 0.26	12.68 ± 0.28
	26	11.13 ± 0.24	11.45 ± 0.30

*Represents the mean of gingival measurements of all teeth (first summed for each tooth then averaged among all teeth of all patients).

were excluded for inability to maintain appointments, thus, 25 patients who completed the study were included. Patient demographics are summarized in Table 1. The proportion of males was twice that of females, with a mean age of 17.48 yr. Mean age between treatment groups was not significantly different (Table 2). All patients at CCHMC were taking a calcium-channel blocker (nifedipine or amlodipine); whereas CCF patients were not receiving other agents contributing to gingival overgrowth. No difference was noted between these two groups.

Table 3 contains actual and adjusted mean of gingival sulcus measurements correlating to overgrowth. The adjusted mean represents adjustment for other effects in the model such as time, medication, and number of patients; and provides more precise calculations.

Baseline gingival measurements were not statistically different between the azithromycin vs. metronidazole treatment groups (12.87 ± 0.40 mm vs. 13.13 ± 0.29 mm, respectively). After treatment, the mean measurement in azithromycin-treated patients across all time points was 11.50 ± 0.14 mm. The mean gingival measurement in metronidazole-treated patients post-treatment was 12.23 ± 0.12 mm. A significant interaction between drug and time showed azithromycin to have greater overall impact on gingival overgrowth over time ($p < 0.001$).

Table 4. Evaluation of efficacy of treatment of gingival overgrowth within each drug group

	Azithromycin		Metronidazole	
Comparison	Mean difference ± s.e. (mm)	p-value	Mean difference ± s.e. (mm)	p-value
Baseline vs. 2 wk	1.60 ± 0.16	<0.0001*	0.86 ± 0.15	<0.0001*
Baseline vs. 4 wk	1.55 ± 0.23	<0.0001*	−0.03 ± 0.20	0.8773
Baseline vs. 6 wk	1.49 ± 0.26	<0.0001*	0.93 ± 0.23	<0.0001*
Baseline vs. 12 wk	1.61 ± 0.35	<0.0001*	0.44 ± 0.31	0.1489
Baseline vs. 26 wk	1.51 ± 0.42	0.0004*	1.68 ± 0.38	<0.0001*
2 wk vs. 4 wk	−0.05 ± 0.18	0.7970	−0.89 ± 0.15	<0.0001*
4 wk vs. 6 wk	−0.06 ± 0.18	0.7509	0.96 ± 0.15	<0.0001*
6 wk vs. 12 wk	0.12 ± 0.29	0.6796	−0.49 ± 0.25	0.0536
12 wk vs. 26 wk	−0.11 ± 0.37	0.7750	1.23 ± 0.33	0.0002*

*Statistically significant ($p < 0.05$).

Table 5. Comparison of efficacy of treatment of gingival overgrowth between groups

	Azithromycin vs. metronidazole	
Time interval (wk)	Mean difference ± s.e. (mm)	p-value
Baseline	−0.26 ± 0.41	0.5289
2	−1.00 ± 0.41	0.0154*
4	−1.84 ± 0.42	<0.0001*
6	−0.82 ± 0.41	0.0474*
12	−1.43 ± 0.42	0.0007*
26	−0.09 ± 0.45	0.8422

*Statistically significant ($p < 0.05$).

Pairwise differences are included in Tables 4 and 5. The azithromycin-treated group showed significant improvement across all time intervals when compared with baseline; the metronidazole-treated group showed significant improvement only at selected time points. When the two groups were compared at each point (Table 5), greatest improvement was noted at 4 wk when the azithromycin group was significantly better. Photographs of each patient taken at each visit were evaluated by the dentist to evaluate clinical improvement (Fig. 1).

Discussion

Gingival overgrowth, associated with CyA use, has been shown to be more prevalent in children and adolescents. In fact, studies have shown functionally distinct fibroblast subtypes within the gingiva in patients with medication-induced overgrowth (13). A number of risk factors affecting periodontal health in general, such as age, history of previous disease, genetic predisposition, systemic diseases, and osteoporosis may

Fig. 1. Gingival overgrowth showing pretreatment and 6 weeks post-therapy in patient who received therapy with azithromycin [(a) pre-therapy; (b) post-therapy] and patient who was treated with metronidazole [(c) pre-therapy; (d) post-therapy].

be related to gingival overgrowth. Anything that interferes with host response such as immunosuppressive agents, which can permit bacterial overgrowth, must be considered a risk. There seems to be an association between poor oral hygiene and worsening overgrowth.

CyA is a cyclic peptide of fungal origin, which is a powerful immunosuppressant. It is often used with other medications to prevent rejection in transplant patients or for other immune-mediated medical conditions. It is a calcineurin inhibitor, which impairs the immune system by affecting T-helper and T-suppressor lymphocytes (1). Available in oral and intravenous forms, it is extensively distributed with high concentrations in the liver, pancreas, lungs, and fat. It is hepatically metabolized (cytochrome P-450 system) and excreted in bile. Adverse reactions include hypertension, renal toxicity, hepatic toxicity, hirsuitism, and gingival overgrowth (1). Serum trough CyA levels were monitored at each visit and were maintained at each patient's pretherapy levels. Nobody had a significant alteration in serum CyA level.

Azithromycin, a semisynthetic derivative of the macrolide erythromycin, possesses good activity against common gram-positive and some gram-negative pathogens (14). It has been approved by the Food and Drug Administration for pediatric use and is available in intravenous or oral preparation. Oral absorption is rapid with 37% bioavailability and it is not thought to affect hepatic enzyme metabolism (14–16). Drug concentrations in mucosal tissues such as the tonsils and sinuses exceed those in the serum (15). Slow elimination from the tissues allows extended tissue concentrations. Adverse effects include diarrhea, nausea, abdominal pain, and vomiting (16–18); however in our study, no patients experienced side effects.

Metronidazole is a synthetic antibacterial and antiprotozoal agent, which is effective against many microbials including anaerobes, *Trichomonas*, and *Clostridium* species. It is well absorbed orally and metabolized in the liver. Adverse reactions include dizziness, headache, nausea, diarrhea, loss of appetite, metallic taste, peripheral neuropathy, constipation, and vomiting (19). In our study, one patient experienced headaches and two patients complained of metallic taste. Although prior case reports (10) have implicated an improvement in gingival

overgrowth using metronidazole, Aufricht et al. (20) showed no improvement with metronidazole given for 7 days using a total dose of 750 mg (10–25 mg/kg) divided three times a day. The authors did note that all patients noticed improvement in gingival bleeding tendency. Our study showed that metronidazole can improve CyA-induced gingival overgrowth. We utilized a higher total daily dose (45 mg/kg/day) than the dose used by Aufricht et al., which may be the cause for improvement in gingival overgrowth.

Both medications were shown to be effective in reducing the amount of gingival overgrowth; however, azithromycin had a more consistent impact. When the baseline gingival measurements were compared with interval post-therapy measurements, there was sustained improvement in gingival overgrowth through the entire 26-wk time period. Patients in the metronidazole group did show improvement when measurements were compared between baseline and the 26-wk visits. However, the improvement was not as great, nor was it consistent. For example, the patients improved from the baseline to the 2-wk visit, however, had worsening of their overgrowth between the 2 and 4-wk intervals. This worsening in overgrowth was also seen in the 6–12 wk interval within the same group of patients (Table 4).

When the two groups of patients were compared with one another, the azithromycin-treated group had significantly greater improvement in gingiva at the 4–12 wk visits than the metronidazole group, again, favoring the use of azithromycin over metronidazole for the treatment of CyA-induced gingival overgrowth (Table 5).

Patients often reported improvement in their gingival health and these subjective observations can be related temporally to the objective data recorded by the examiners. Several patients in the azithromycin group relayed a decrease in gingival bleeding and greater ease of brushing. One patient stated his appetite greatly improved as he felt, he could chew his food with much greater ease. Clinical photographs are depicted in Fig. 1.

In summary, treatment of CyA-induced gingival overgrowth is possible with antibacterial agents. Although metronidazole offers improvement in gingival overgrowth, the results of this study show azithromycin therapy to be superior. Consequently, gingival overgrowth alone is not a valid justification to discontinue CyA therapy.

Acknowledgments

The authors would like to thank the dental hygienists and staff photographers at CCF for their technical assistance; and Suzanne Southerland MD, Faith Nkosi DMD, and Pam Hutchinson RN, CNN for their assistance with data collection.

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