J Periodont Res 2003; 38; 141–146
Printed in the UK. All rights reserved

JOURNAL OF PERIODONTAL RESEARCH
ISSN 0022-3484

Antibiotic treatment of incipient drug-induced gingival overgrowth in adult renal transplant patients

F. L. Mesa[1], A. Osuna[2], J. Aneiros[3], M. Gonzalez-Jaranay[1], J. Bravo[2], P. Junco[1], R. G. Del Moral[3], F. O'Valle[3]

[1]Department of Periodontics, School of Dentistry, University of Granada, [2]Department of Nephrology, Virgen de las Nieves University Hospital of Granada, [3]Department of Pathology, University Hospital, School of Medicine, University of Granada, Granada, Spain

Mesa FL, Osuna A, Aneiros J, Gonzalez-Jaranay M, Bravo J, Junco P, Del Moral RG, O'Valle F. Antibiotic treatment of incipient drug-induced gingival overgrowth in adult renal transplant patients. J Periodont Res 2003; 38; 141–146.

Background: Drug-induced gingival overgrowth (GO) remains a challenge in periodontics. Partial and total regressions of this GO have been reported after a short course of antibiotics.

Methods: We conducted a double-blinded controlled randomised study to determine the effect of metronidazole (MNZ) or azithromycin (AZM) on the regression of incipient cyclosporin A-induced GO in 40 adult renal transplanted patients. The quantitation of the GO was performed with Image Digital Analysis.

Results: None of the patients with GO showed complete remission after 30 days. The pretreatment GO index was 0.895 ± 0.16 in the metronidazole treatment group (MNZ group, $n = 13$), 0.932 ± 0.11 in the azithromycin treatment group (AZM group, $n = 14$), and 1.073 ± 0.32 in the controls (placebo group, n=13). At the end of the study (30 days), the GO index score was lower in 54.4% and 62.3% of the MNZ and AZM groups, respectively, and the mean score differences were statistically significant between the groups (0.897 ± 0.28, MNZ group vs. 0.909 ± 0.15, AZM group vs. 1.130 ± 0.3, placebo group, $P < 0.05$ ANOVA).

Conclusions: A 7-day course of MNZ or AZM does not induce remission of CsA-induced GO, although it acts on concomitant bacterial over-infection and gingival inflammation.

Francisco O'Valle, Avda. de Madrid n°11, Facultad de Medicina, Dpto. de Anatomía Patológica., 18012 Granada, Spain
Tel: +34 958243509
Fax: +34 958243510
e-mail: fovalle@ugr.es

Key words: azithromycin, cyclosporin A, gingival overgrowth, metronidazole

Accepted for publication January 31, 2001

Gingival overgrowth (GO) is a widely documented side-effect of cyclosporin A (CsA), which is treated with oral hygiene measures for plaque control and with partial gingivectomy in severe cases (1).

Although the exact pathogenesis and histopathology of CsA-induced GO have still to be fully defined, clinically detected GO is a combination of two principal histological components: fibrotic enlargement of drug origin and inflammatory lesion due to microbial dental plaque (2, 3).

In the last few years, various case reports have described the utility of short courses of metronidazole (MNZ) or azithromycin (AZM) for the partial or complete remission of drug-induced GO (4–9). MNZ is particularly active against anaerobes associated with periodontitis, especially spirochetes (10), and against *Actinobacillus actinomycetemcomitans* present in the bacterial plaque (11). In association with spiramycin, MNZ acquires a high concentration and long half-life in the gingival crevicular fluid, important for the elimination of periodontopathogenic bacteria (3, 12).

Reported results of the antibiotic treatment of CsA-induced GO are not totally consistent. Wong *et al.* (4) obtained the complete remission of this GO in four young women with kidney transplant after 1.2 g/day MNZ during 1 week. More recently, Aufircht *et al.* (13) reported that MNZ treatment produced no improvement in CsA- induced GO among paediatric transplant patients, especially among those receiving adjuvant calcium

channel blocker therapy. On the other hand, AZM treatment produced a decrease in gingival sulcus depths and in lengths of teeth and interdental papillae to the cementum–enamel junction, with patients reporting amelioration in gum bleeding and two-thirds of them describing the treatment as at least somewhat useful (14). Gómez *et al*. (6) reported improvement in 27 patients with GO, observing a greater degree of improvement in the patients with lower baseline GO scores.

The aim of the present study was to determine the effect of MNZ and AZM on incipient GO secondary to treatment with CsA (Sandimmum®, Novartis, Basel, Switzerland) in adult renal transplanted patients by means of a controlled clinical study, using Image Digital Analysis for the quantitation of the GO.

Patients and methods

A double-blind controlled randomised study was conducted in order to determine the effect of treatment with MNZ or AZM in kidney transplant patients with CsA-induced incipient GO. All of the patients were recruited from our hospital. The study received the approval of the Ethical Committee of our hospital.

Inclusion criteria for this clinical study were:

- to be a receptor of a renal transplant;
- to be older than 18 years;
- for the time elapsed from the transplant to be > 6 months and < 2 years;
- to be receiving CsA;
- to retain at least four incisor teeth in both mandible and maxilla;
- to have not undergone professional tooth cleaning immediately before or during the treatment;
- to have provided written informed consent to participate in the study.

Exclusion criteria were:

- presence of intense acute gingivitis;
- clinical suspicion of allograft rejection, or;
- pharmacological interaction between the drugs received and MNZ or AZM.

Fourteen patients were excluded from the initial study population of 54 patients (three patients refused to participate in the clinical study; two had intense acute gingivitis; four did not retain four incisor teeth in both mandible and maxilla, and five underwent professional tooth cleaning immediately before the treatment), leaving a final study group of 40 patients, who were randomly assigned to one of three groups by drawing one of three different-coloured balls from a bag. The two experimental groups were: MNZ group ($n = 13$), who received 250 mg metronidazole 3 times daily for 7 days; and AZM group ($n = 14$), who received azithromycin 500 mg twice daily for 7 days. A placebo group ($n = 13$) received one placebo capsule 3 times daily for 7 days. Two observers (F.L.M.A., M.G.J.) carried out the oral examination of all of the patients.

The mean age of the patients was 41.4 years (range 24–60). The following data were collected on each patient:

- oral indexes (plaque index and bleeding index);
- clinical assessment of the GO following the criteria of Pernu *et al*. (15);
- morphometric GO index (GOI) (16);
- sex;
- age;
- time lapsed from the transplant;
- treatment with calcium channel blockers;
- renal function (serum creatinine and creatinine clearance), and;
- blood CsA levels.

At the beginning of the study, an intraoral photo was taken of each patient, using an F-3 camera (Nikon, Tokyo, Japan) with a 120 mm telephoto lens and ring flash (Nikon), at a constant distance of 0.45 m and using a mouth-opening device. We used 100 ASA black-and-white film (Ilford, Cheshire, UK). The development was done on 13 × 9 grade 3 polythermized paper (Ilford). Thirty days later, the intraoral photograph was repeated in all patients under the same conditions.

The modifications induced in the GO were assessed by means of image digital analysis (IDA). For this purpose, the b/w photographs were digitised using an Agfa Arcus II scanner with Arcus Bonus software (Agfa, Barcelona, Spain). The quantitation was done using the Visilog 4.1 image analysis software (Noesis CORP., Velizy, France) in a Windows 95 environment (Microsoft Corporation, Redmond, WA, USA). The surfaces measured at the beginning and at the end of the study were the gingival area (GA) and dental area (DA). The relationship between them was considered the gingival overgrowth index (GOI = GA/DA), following the methodology previously described by our group (16). The automatic quantitation of these areas by IDA allowed us to establish objectively the post-treatment modifications.

The data obtained were exported to the RSigma Babel database (Horus, Harware, Barcelona, Spain). The Kolmogorov–Smirnov test was used to determine whether the variables were adjusted to a normal distribution. Once this was demonstrated, one-way analysis of variance tests (ANOVA) and independent means comparisons (Student's *t*-test) were applied.

Results

Tables 1 and 2 show the values and distribution of some of the clinical, analytic and morphometric variables studied. The mean patient age and gender distributions were similar in the three groups (39.1 ± 11 years old in the MNZ group, 41.9. ± 14 years old in the AZM group, and 43.3 ± 10 years old in the placebo group) (Table 1). Regarding the renal function at the beginning of the study, the patients of the MNZ group presented significantly higher serum creatinine levels than those of the placebo group (3.64 ± 2.63 vs. 1.60 ± 0.57, respectively, $p < 0.05$ Student's *t*-test), which decreased after the 30 days of treatment (Table 1). The serum levels of CsA were similar in the three groups and were within the therapeutic range in all cases (MNZ group 170.5 ± 27, AZM group 178.2 ± 20, placebo group 175.8 ± 29).

The degree of oral health in the three groups was also similar at the beginning of the study. The patients had poor oral health, with medium index values and very wide ranges (plaque

Table 1. Clinical and analytical data from clinical study of kidney transplant patients

	Day 0				Day 30				
	MNZ ($n = 18$)	AZM ($n = 18$)	Placebo ($n = 18$)	Total n (%)	MNZ ($n = 13$)	AZM ($n = 14$)	Placebo ($n = 13$)	Total n (%)	P-value
Age*	43.1 ± 14	45.9 ± 12	41.7 ± 9		39.1 ± 11	41.9 ± 14	43.3 ± 10		NS
Gender									
Male	10	9	8	27 (50%)	7	6	6	19 (47.5%)	
Female	8	9	10	27 (50%)	6	8	7	21 (52.5%)	
Serum creatinine (mg/dL)*	3.64 ± 2.63	2.3 ± 1.75	1.60 ± 0.57		2.90 ± 1.61	2.01 ± 2	1.56 ± 0.49		$P < 0.05$
Creatinine clearance ($mg\%{\cdot}mL^{-1}{\cdot}min^{-1}$)*	140.12 ± 21.17	54.90 ± 21.33	74.90 ± 26.23		41.37 ± 20.39	56.61 ± 23.18	75.04 ± 25.11		$P < 0.01$
Blood CsA levels (ng/mL)*	170.50 ± 27.0	178.20 ± 20.0	175.80 ± 29.0		168.30 ± 25.0	165.10 ± 15.0	170.61 ± 27.0		NS

*The values correspond to the mean ± standard deviation. P-value: level of significance between groups at 0 and 30 days, ANOVA test. NS: Not significant.

Table 2. Morphometric data of gingival overgrowth from clinical study of kidney transplant patients

Gingival overgrowth*	Day 0				Day 30				
	MNZ ($n = 13$)	AZM ($n = 14$)	Placebo ($n = 13$)	Total n (%)	MNZ ($n = 13$)	AZM ($n = 14$)	Placebo ($n = 13$)	Total n (%)	P-value
Scoring 0	4 (0.843 ± 0.19)	5 (0.821 ± 0.14)	4 (0.850 ± 0.14)	13 (32.5%)	4 (0.836 ± 0.23)	5 (0.820 ± 0.18)	4 (0.918 ± 0.14)	13 (32.5%)	NS
Scoring 1	7 (0.932 ± 0.18)	6 (0.943 ± 0.20)	5 (0.962 ± 0.22)	18 (45.0%)	8 (0.906 ± 0.35)	7 (0.923 ± 0.13)	5 (1.121 ± 0.27)	20 (50.0%)	NS
Scoring 2	2 (0.910 ± 0.11)	3 (1.033 ± 0.17)	4 (1.408 ± 0.29)	9 (22.5%)	1 (0.951 ± 0.15)	2 (0.984 ± 0.16)	4 (1.360 ± 0.34)	7 (17.5%)	NS
Scoring 3	0 (–)	0 (–)	0 (–)	0 (0%)	0 (–)	0 (–)	0 (–)	0 (0%)	–

*The values correspond to the number of patients for each score, with morphometric data in brackets (mean ± standard deviation). P-value: level of significance between groups at 0 and 30 days, ANOVA test. NS: Not significant.

index: 51.76%, range 10–100%; bleeding index 54.57%, range 0–100%). The MNZ and AZM groups showed a slight decrease in the oral health index scores after the treatment (mean plaque index: 35.61% and 32.50%, mean bleeding index: 42.45% and 39.40%, respectively), whereas there was no reduction in the placebo group (mean plaque index: 53.23%, mean bleeding index: 56.35%).

The patient distribution according to the initial clinical GO assessment was as follows:

- 32.5%, no clinical signs of GO;
- 45.0%, GO grade 1;
- 22.5%, GO grade 2.
- No patient presented clinical GO grade 3 (Table 2).

At baseline, the overall GOI scores according to the clinical GO were as follows:

- no clinical signs of GO, GOI of 0.838 ± 0.16;
- GO grade 1, GOI of 0.945 ± 0.19;
- GO grade 2, GOI of 1.117 ± 0.35.

The mean baseline GOI was 0.895 ± 0.16 in the MNZ group, 0.932 ± 0.11 in the AZM group and 1.073 ± 0.32 in the placebo group. After the treatment and 30 days of evolution, the GOI of the MNZ and AZM groups had not changed (0.897 ± 0.28 and 0.909 ± 0.15, respectively), whereas the GOI of the placebo group showed a non-significant tendency to increase. Although there were no differences in the baseline GOI of the three groups, the GOI increase in the placebo group resulted in a statistically significant difference in GOI between each experimental group and the placebo group at the end of the study (1.130 ± 0.3, placebo group, vs. 0.897 ± 0.28, MNZ group and 0.909 ± 0.15, AZM; $P < 0.05$ ANOVA test) (Fig. 1). The difference was greater when patients with grades 1 and 2 clinical GO were compared at the end of the study (1.24 ± 0.31, placebo group vs. 0.92 ± 0.32, MNZ group and 0.953 ± 0.26, AZM group; $P < 0.05$ ANOVA test).

At the end of the study (day 30), the GOI was lower in 54.4% of the MNZ and 62.3% of the AZM groups, whereas it was lower in only 23.1% of the patients that received placebo and with

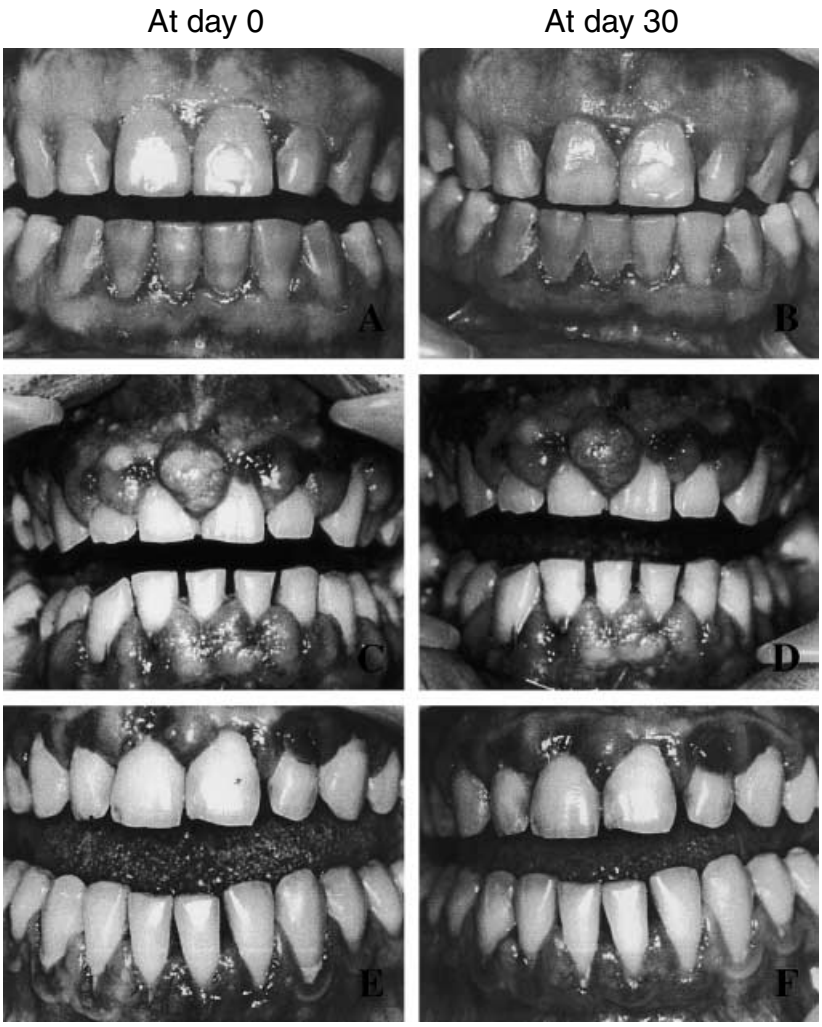

Fig. 1. Transplanted renal patients with different grades of CsA-induced gingival overgrowth. Photos A and B: placebo group patient presented grade 1 GO with gingivitis by plaque at day 0 (A), which increased at day 30 (B) in interdental papillae 22–23, 32–33 and 43–44. Photos C and D: metronidazole group patient presented grade 2 GO at day 0 (C) and at day 30 presented slight reduction in interdental papillae 21–22 and 22–23, with a small reduction in mandibular central papillae (D). Photos E and F: azithromycin group patient presented grade 1 GO at day 0 (E) and at day 30 a small increase in gingival margin of 22 and interdental papillae 21–22 but slight reduction in width of mandibular interdental papillae (F). Bar = 0.5 cm.

a smaller difference. At the end of the study, none of the 27 patients in the study with GO showed complete remission.

Discussion

CsA-induced GO occurs in a variable percentage (20–70%) of the patients who receive CsA therapy (1). Mild or moderate GO was observed in 67.5% of the 40 patients in the present study, who were consecutively evaluated by periodontists > 12 months after the transplant. These data reveal the need to control the patient's periodontal condition, described by Seymour *et al.* (17) as the only risk factor that the periodontist can influence.

The pathogeny of CsA-induced GO has not been fully established but is recognized to be multifactorial, with inflammatory phenomena playing an important role (18, 19). About 40% of CsA-induced GO can be considered inflammatory in nature (3) and it has been hypothesized that the fibrotic enlargement due to drug therapy is magnified by the oedematous component, resulting in an apparent increase of the clinical GO (3). The gingival lamina propria of transplant patients invariably contains inflammatory infiltrate, despite the blocking of the immune response. The intensity of this CD45-positive infiltrate is significantly related to the severity of the GO (16, 19), as is also an increase in CD68-positive macrophages in the lamina propria (16). The macrophages are the main producers of TGF-β1, the cytokine responsible for the fibrous hyperplastic response. In addition, CsA induces alterations in the cytokine profile in gingival tissue and inflammatory infiltrate (increase of IL-1β, IL-6 interleukins) that cause dysregulation of the connective tissue turnover, with a resultant accumulation of matrix components (20).

Most studies show an association between oral hygiene status and the prevalence and severity of drug-induced gingival overgrowth (1). This suggests that plaque-induced gingival inflammation may be important in the development and expression of the gingival changes. The changes in gingival contour seen in drug-induced GO may be at least exacerbated by plaque-induced inflammation (19). All of the above explains the better outcomes achieved when there are improvements in oral hygiene alongside the antibiotic treatment. Our outcomes might have been better if we had eliminated local irritants and local inflammation with scaling and oral hygiene instructions, but the aim of our study was to assess the drug effect alone.

Antimicrobial therapy with macrolides plays a prominent role in the treatment of acute odontogenic infections and is also used as adjuvant or prophylactic treatment in the surgical therapy of periodontal infection associated with chronic inflammatory processes (21). MNZ has been shown to have a pronounced effect on the subgingival microbiota of periodontal lesions in humans and, after multiple doses, to reach a high concentration in the gingival crevicular fluid, in excess of that regarded as the minimal inhibitory concentration for most periodontal pathogens (22).

The results of our controlled study on adult patients indicate that MNZ treatment does not induce remission of GO in incipient cases. This finding contradicts the results of a study by Wong *et al*. (4), who reported complete remission in four patients with GO, in some cases severe, and control of GO recurrence, using a similar treatment protocol to ours. Their patients may have presented a GO with an exacerbated inflammatory component or one that was not truly drug induced but rather of an inflammatory or gingivitis type in relation to its immunosuppression status. Consistent with the present results, Aufircht *et al*. (13) reported no improvement in CsA-induced GO among paediatric transplant patients treated with MNZ, especially among those receiving adjuvant calcium channel blocker therapy. Our experiment showed that a short course of MNZ does not induce remission of CsA-induced GO, as demonstrated by the small difference in gingival area comparing the second intraoral image of the treatment group with the first. A reduction in the GOI was obtained in 54% of the MNZ-treated patients, and the mean GOI of the MNZ-treated group was significantly lower than that of the placebo group at the end of the study. These findings probably reflect the antibacterial effect of MNZ, which can be confused with a partial abatement of the progressive clinical course of the GO. MNZ is metabolised by cytochrome P_{450} and is eliminated renally, and thus does not interfere with the serum levels of CsA (4). In our patients, the initial creatinine levels were higher than those found in normal subjects and the MNZ group presented an even worse renal function. The creatinine and creatinine clearance values were not significantly changed at the end of the study, indicating that MNZ is not nephrotoxic and does not interfere with the renal function.

Recently published studies used AZM to induce partial and at times complete remission of this GO (6–9, 14). AZM is a semisynthetic, acid-stable antibiotic of a novel class of macrolides named azalides derived from erythromycin, which does not affect hepatic cytochrome P_{450} and does not modify the pharmacokinetics of other drugs, including CsA (23). The special structure provides acid stability and particular pharmacokinetic properties. Indeed, after oral intake, AZM can be detected in gingival tissue for 7–10 days (21) and AZM concentrations in saliva, normal gingiva and pathological periodontal tissues are 10–100-fold those in plasma, indicating that AZM is retained in these target compartments (24). Moreover, AZM rapidly penetrates both fibroblast and phagocytic cells, where the concentrations are 100–200-fold those found in extracellular compartments (24).

Gómez *et al*. (6) reported improvement in 27 patients with CsA-induced GO treated with AZM and found that the degree of improvement was better when the GO score was lower. Palomar *et al*. (7) used AZM to treat 10 renal transplant patients with gingival overgrowth, achieving remission in five cases and an improvement of > 50% in the other five. Nowicki *et al*. (9) reported the partial regression of severe GO (grade 3) in a renal transplant patient treated with CsA after 3 days of AZM treatment, although a repeat dose after 3 months produced no further regression. None of these studies used control groups but rather simply described the clinical findings, with no exhaustive study of the oral cavity. Nash & Zaltzman (14) reported significant improvements in gingival sulcus depth, tooth length, and gum bleeding after AZM treatment but there was no evaluation of gingival biopsy to confirm modifications in histopathologic lesions or inflammatory status. Unlike these works, our study found only a slight reduction in the GOI of AZM-treated patients, although this was significantly better than the GOI of the placebo group at the end of the study.

We believe that all of the above outcomes could be related to a reduction in the accompanying inflammatory process rather than to a modification of the fibrogenic process in the gingival lamina propria. The IDA quantitation proposed by our group (16) and by Ellis *et al*. (25) yields a more accurate measurement than does clinical evaluation for the purposes of testing the effects of drug therapy.

At the oral examination, which should be performed by a specialist dentist, a distinction should be drawn between genuine GO and inflammatory processes to avoid confusion in interpreting the real effects of antibiotic treatment. Given current concerns about the excessive use of antibiotics in this and other settings, it is important to minimise the inappropriate administration of these drugs.

In conclusion, a short course of MNZ or AZM does not produce a regression of CsA-induced GO, although it acts on concomitant bacterial overinfection and gingival inflammation.

Acknowledgements

The authors are grateful to Inmaculada Morillas for the intraoral photography and to Richard Davies for assistance with the English version.

References

1. Daley TD, Wysocki GP. Clinical and pharmacologic correlations in cyclosporine-induced gingival hyperplasia. *Oral Surg Oral Med Pathol* 1986;**62:**417–421.
2. Hassell TM, Hefti AF. Drug-induced gingival overgrowth: Old problem. *Crit Rev Oral Biol Med* 1991;**2:**103–107.
3. Kantarci A, Cebeci I, Tuncer O, Carin M, Firatli E. Clinical effects of periodontal therapy on the severity of cyclosporin A-induced gingival hyperplasia. *J Periodontol* 1999;**70:**587–593.
4. Wong W, Hodge MG, Lewis A, Sharpstone P, Kingswood JC. Resolution of cyclosporin-induced gingival hypertrophy with metronidazole. *Lancet* 1994;**343:**989.
5. Wahlstrom E, Zamora JU, Teichman S. Improvement in cyclosporin-associated gingival hyperplasia with azithromycin therapy. *N Engl J Med* 1995;**332:**753–754.
6. Gómez E, Sánchez-Núnez M, Sánchez JE, *et al*. Treatment of cyclosporin-induced gingival hyperplasia with azithromycin. *Nephrol Dial Transplant* 1997;**12:**2694–2697.
7. Palomar R, Belart M, Soy D, *et al*. Effectiveness and safety of azithromycin in the treatment of cyclosporine-induced gingival overgrowth. *Nephron* 1998; **79:**101–102.
8. Jucgla A, Moreso F, Sais G, *et al*. The use of azithromycin for cyclosporin-induced gingival overgrowth. *Br J Dermatol* 1998;**138:**188–189.

9. Nowicki M, Kokot F, Wiecek A. Partial regression of advanced cyclosporin-induced gingival hyperplasia after treatment with azithromycin. A case report. *Ann Transplant* 1998;**3:**25–27.
10. Lesche WJ, Grossman N, Giordano J. Metronidazole in periodontitis. *J Clin Periodontol* 1993;**20:**96–104.
11. Pavicic MJ, van Winkelhoff AJ, Douque NH, Steures RW, de Graaff J. Microbiological and clinical effects of metronidazole and amoxicillin in *Actinobacillus actinomicetemcomitans*-associated periodontitis. *J Clin Periodontol* 1994;**21:**107–112.
12. Rotzetter PA, Le Liboux A, Pichard E, Cimasoni G. Kinetics of spiramycin/metronidazole (Rodogyl) in human gingival crevicular fluid, saliva and blood. *J Clin Periodontol* 1994;**21:**595–600.
13. Aufricht C, Hogan EL, Ettenger RB. Oral metronidazole does not improve cyclosporine A-induced gingival hyperplasia. *Pediatr Nephrol* 1997;**11:**552–555.
14. Nash MM, Zaltzman JS. Efficacy of azithromycin in the treatment of cyclosporine-induced gingival hyperplasia in renal transplant recipients. *Transplantation* 1998;**65:**1611–1615.
15. Pernu HE, Pernu LM, Knuuttila ML, Huttunen KR. Gingival overgrowth among renal transplant recipients and uraemic patients. *Nephrol Dial Transplant* 1993;**8:**1254–1258.
16. 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.
17. Seymour RA, Ellis JS, Thomason JM. Risk factors for drug-induced gingival overgrowth. *J Clin Periodontol* 2000;**27:**217–223.
18. Mesa FL, López C, Moreno E, O'Valle F. Crecimientos gingivales inducidos por medicamentos. *Periodoncia* 1994;**4:**177–186.
19. Seymour RA, Thomason JM, Ellis JS. The pathogenesis of drug-induced gingival overgrowth. *J Clin Periodontol* 1996;**23:**165–175.
20. Myrillas TT, Linden GJ, Marley JJ, Irwin CR. Cyclosporin A regulates interleukin-1β and interleukin-6 expression in gingiva: implications for gingival overgrowth. *J Periodontol* 1999;**70:**294–300.
21. Van Oosten MAC, Notten FJW, Mikx FHM. Metronidazole concentrations in human plasma, saliva, and gingival crevice fluid after a single dose. *J Dent Res* 1986;**65:**1420–1423.
22. Amacher DE, Schomacker SJ, Retsema JA. Comparison of the effects of the new azalide antibiotic, azithromycin estolate on rat liver cytochrome P-450. *Antimicrob Agents Chemother* 1991;**35:**1186–1190.
23. Foulds G, Johnson RB. Selection of dose regimens of azithromycin. *J Antimicrob Chemother* 1993;**31:**39–50.
24. Gladue RP, Snyder ME. Intracellular accumulation of azithromycin by cultured human fibroblasts. *Antimicrob Agents Chemother* 1990;**34:**1056–1060.
25. Ellis JS, Seymour RA, Robertson P, Butler TJ, Thomason JM. Photographic scoring of gingival overgrowth. *J Clin Periodontol* 2001;**28:**81–85.