SIDE EFFECTS OF CYCLOSPORINE-A TREATMENT IN RATS: GINGIVAL OVERGROWTH AND EARLY HYPERGLYCEMIA

Orlando L. Catanzaro[1,2], Lorena Calvo Lauria[1], Pablo Obregon[2], Emilio S. Labal[1], Ricardo R. Rodriguez[2]

[1] Department of Biochemistry- School of Dentistry, John F Kennedy University.
[2] School of Dentistry and Medicine, University Del Salvador, Argentina.

ABSTRACT

Gingival overgrowth is an adverse side effect of cyclosporine A (CsA) in the treatment of transplanted patients. The purpose of this study was to evaluate the effects of CsA on new-onset diabetes mellitus and gingival overgrowth in rats, by measuring collagen, nitric oxide and microvascular permeability. Blood glucose level, collagen, nitric oxide level and vascular permeability were determined. Blood glucose level increased significantly from 6.5 +/- 0.9 for the control group to 15+/- 1.2, 17 +/- 1.2 and 21.6+/- 1.6 mM/L at 1, 4 or 8 weeks of CsA treatment, respectively. Collagen (ug HO Proline/mg p) increased significantly from 2.5+/- 0.5 for the control group to 4.2+/- 0.8, 5.9+/- 0.6 and 7.3 +/- 0.8 at 1, 4 or 8 weeks of CsA treatment, respectively. Vascular permeability was 10.3+/- 1.2 for the control group and 15+/-1; 17.2 +/- 1.3, and 22.1+/- 2.1 ug EB/g T; at 1, 4 or 8 weeks of CsA treatment, respectively. Nitric oxide level was 3.5 +/- .9 umol/mg P for the control group and 4+/- 0.2, 8.2+/- 0.9 and 11+/-1 for 1, 2 or 8 weeks of CsA treatment, respectively. These findings appear to indicate that the development of significant gingival changes induced by CsA is related to new-onset of diabetes mellitus during the immunosuppressive treatment.

Key words: Cyclosporine A, gingival hypertrophy, hyperglycemia, collagen.

EFECTOS COLATERALES DEL TRATAMIENTO CON CYCLOSPORINA-A EN RATAS: HIPERPLASIA GINGIVAL E HYPERGLUCEMIA TEMPRANA

RESUMEN

La hiperplasia gingival es un efecto colateral adverso del tratamiento con ciclosporina A (CsA) en pacientes transplantados. El propósito de este estudio fue evaluar el efecto de CsA en el inicio de diabetes mellitus, la concentración de colágeno, y de óxido nítrico y la permeabilidad capilar gingival. El nivel de glucosa en sangre de los animales controles fue: 6.5+/- 0.9, en tanto que los tratados con CsA fue: 15+/-1.2; 17+/- 1.1 y 21.6+/- 1.6 mM/L a las 1, 4 y 8 semanas respectivamente. El colágeno (ug OH prolina/mg p) mostró un aumento significativo en los animales tratados con CsA respecto de los controles: 2.5+/- 0.5; 4.2+/- 0.8; 5.9+/- 0.6; 7.3+/- 0.8 respectivamente a las 1,4 y 8 semanas de tratamiento. Los valores de permeabilidad capilar (ug AE/ g T) fueron: en los animales control 10.3+./- 1.2; en los animales tratados con CsA, a las 1, 4 y 8 semanas 15+/- 1.0; 17.2 +/- 1.3 y 22.1+/- 2.1 respectivamente. Los valores de óxido nítrico (umol/mg p) en los animales control: 3.5+/-0.9; y en los animales tratados con CsA 4+/- 0.2; 8.2+/- 0.9 y 11.2 +/- 1.0 respectivamente. Estos resultados parecen indicar que el desarrollo de los significativos cambios gingivales inducidos por la administración de CsA está relacionado con la hiperglucemia temprana que se asocia al tratamiento con inmunosupresores.

Palabras clave: Ciclosporina A, hipertrofia gingival, hiperglucemia, colágeno.

INTRODUCTION

One of the adverse effects of Cyclosporine A (CsA), which occurs in up 70% of the patients receiving it, is the development of gingival overgrowth. The use of cyclosporine A as an immunosuppressant has revolutionized organ transplant, which has become the management of choice for many patients with chronic and life-threatening conditions[1,2]. It is not surprising that the administration of this drug is often associated with several side effects. These include nephropathy[3,4], hepatotoxicity[5,6], neurotoxicity[7], lymphoproliferative neoplasm[8,9] and gingival overgrowth[10]. Procedures to block islets autoimmunity in diabetes type 1 have been proposed as effective means to prevent the disease, and agents such CsA have demonstrated that immunosuppresion can reduce insulin requirements in patients with recent-onset type 1 diabetes[11,12]. However new-onset diabetes mellitus after organ transplantation has been recently described[13,14]. The aim of this study was to determine the effects of CsA on gingival alterations and new-onset of diabetes mellitus as a risk factor for gingival enlargement.

MATERIALS AND METHODS

Male albino Wistar rats weighing 200-250 g were used. They were randomly distributed into 4 groups of 8 animals each. The experimental protocol was approved by the local Institutional Committee for Animal care and use of University del Salvador.

Group I: Control rats,received physiological saline; Groups II, III and IV: rats were injected subcutaneously with a daily dose of 10 mg/ Kg body weight of CsA-vehicule(olive oil) for 1, 4 and 8 weeks.

Biochemical Analysis

After 1, 4 and 8 weeks of treatment, blood samples were obtained from a tail vein for glucose measurement by reagent strips (Accu-Chek, Roche, Argentina). Gingival mucosal tissue was obtained for analytical determinations. Tissue was extracted and washed extensively with 50 mM Tris – HCl (pH 7.5) at 4 C. Tissue was drained on filter paper, weighed and homogenized in cold 10 mM Tris-HCl, pH 8.2; the homogenate was centrifuged (10.000 x g) at 4º C for 30 min and the supernatant saved for biochemical determinations. Protein was determined according to the method of Lowry[15]. Nitrites were determined with Griess reagent (Promega systems) according to Green et al[16]. Collagen was determined according to the method of Walsh et al[17] with slight modification [18]. To assess vascular permeability, animals received Evans Blue (EB) (2.5 %, dissolved in physiological saline, at a dose of 50 mg Kg^{-1} intravenously). At the end of the experiments the excess dye remaining in the gingival mucosal capillaries was removed by retrograde intra-aortic injection of isotonic saline solution. Gingival tissue samples were incubated in formamide (1 ml) for 48 h and then EB content was determined by spectrophotometry at 620 nm.

RESULTS

The average blood glucose concentration was 6.5+/- 0.9 mM/L in the untreated control group at 1, 4 and 8 weeks. On the other hand, for the group of rats with CsA treatment, the values were 15 +/- 1.2; 17+/-1.1 and 21.6+/-1.6 mM/L at 1, 4 or 8 weeks treatment, respectively (P<0.001) (Table 1). Table 1 shows the collagen concentration in control rats: 2.5+/- 0.5 ug OH proline/ mg p, compared to rats treated with CsA, which had 4.2+/-0.8; 5.9+/- 0.6 and 7.3+/- 0.8 (P<0.001). One, 4 or 8 weeks after CsA treatment there was a significant increase in EB extravasation in the gingival mucosal tissue (ug EB/g T). The average value for the control group was 10.3+/- 1.2; while for the groups treated with CsA, average values were: 15+/- 1; 17.2+/-1.3 and 22.1+/- 2.1 (P<0.001) at 1, 4 or 8 weeks, respectively (Table 1). In rats with no prior treatment with CsA, the nitric oxide level in gingival tissue was 3.5+/- 0.9 umol NO2/mg P, while the CsA treated rats at 1, 4 or 8 weeks showed marked nitric oxide levels, with values of 4+/- 0.2; 8.2+/- 0.9 and 11.2+/-1, respectively, (P<0.001) (Table 1).

Statistical Analysis

Data are expressed as mean +/- SEM and analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Values of P<0.05 were considered statistically significant.

DISCUSSION

The present study identifies and explores possible risk factors to both the prevalence and severity of drug-induced gingival overgrowth (GO) on gingival response associated to new-onset diabetes mellitus. Age has been reported as a risk factor for cyclosporine-induced GO[19]. Administration of CsA in rats for 60 and 120 days resulted in evident GO[20]. Diabetes increase levels of inflammatory markers C-

Table 1: Effect of CsA treatment on gingival tissue of the rats at different experimental periods.

Treatment	Periods (Wks)	Glycemia (mM/L)	Collagen (ugHO Pro/mgP)	NO (umol/mgP)	Vasc. Perm. (ugEB/gT)
Control		6.5 ± 0.9	2.5 ± 0.5	3.5 ± 0.9	10.3 ± 1.2
CsA	1	15.0 ± 1.2*	4.2 ± 0.8*	4.0 ± 0.2*	15.0 ± 1*
CsA	4	17.0 ± 1.1*	5.9 ± 0.6*	8.2 ± 0.9*	17.2 ± 1.3*
CsA	8	21.6 ± 1.6*	7.3 ± 0.8*	11.2 ± 1*	22.1 ± 2.1*

Values expressed as Mean ± SEM; n=8 rats; P < 0.05 statistical significance vs. control rats; CsA: Cyclosporine A

reactive protein[21], as well as the pro-inflammatory cytokines tumor necrosis factor- alpha[22] and interleukin-6[23]. Periodontal disease has been characterized as the sixth complication of diabetes mellitus[24] and some diabetics are at increased risk of periodontitis. On the other hand, diabetic hyperglycemia is a systemic disease positively associated with attachment loss. In this study, we observed that the increased blood glucose concentration after CsA treatment at 1, 4 an 8 weeks produced significant changes in gingival tissue. The prevalence of GO in rats treated with CsA was significantly higher after 8 weeks of treatment. Gingival enlargement as a risk factor in kidney and liver transplant with other immunosuppressants treatment produced variable gingival response in patients taking these drugs[25]. The occurrence of diabetes after organ transplantation is recognized as one of the metabolic consequences of therapy with CsA[26]. Although the exact mechanisms involved in the developments of GO caused by CsA are not known, our results suggest that early hyperglycemia might induce local inflammation, which in turn might exacerbate CsA-induced GO in rats. A number of studies had investigated the direct effects of CsA on extracellular matrix synthesis by fibroblasts, in particular collagen metabolism[27]. There is substantial evidence that this drug acts directly or indirectly on the growth and function of both gingival fibroblasts and collagen via cytokines and growth factors[28,29]. In the present study, we showed that CsA caused marked gingival collagen accumulation after 1, 4 and 8 weeks of treatment. Current evidence indicates that the accumulation of collagen seen in GO could be explained by a CsA-induced inhibition of collagenolytic activity within the gingival tissue[30]. After CsA administration, inducible and endothelial forms of nitric oxide synthase play a critical role in GO[31]. The present investigation revealed that GO caused by CsA resulted in a marked increase in NO gingival tissue. Alterations in the pathogenesis of diabetic microangiopathy consist of increased capillary pressure, blood flow and endothelial permeability, and can be detected at an early stage of diabetes mellitus[32,33]. Our results showed that CsA develops well-defined gingival capillary permeability that was evaluated in EB extravasation. Several mechanisms related to diabetic angiopathy could explain gingival capillary permeability: the early onset of diabetes due to CsA treatment, and local inflammation and TGF-1 as a key factor for the development of GO.

In conclusion, these findings appear to indicate that the development of significant gingival changes induced by CsA is related to new-onset diabetes mellitus during the immunosuppressive treatment

ACKNOWLEDGEMENTS

We are especially grateful to Lic. Irene Di Martino for technical assistance and UAJFK for financial support.

CORRESPONDENCE

Dr.Orlando L.Catanzaro
Dirección de Investigaciones Bioquimico-Odontologicas
Parral 221, CP1424 Buenos Aires, Argentina
Ocatan50@aol.com

REFERENCES

1. Faulds D, Goa KL, Benfield P. Cyclosporine: A review of pharmacodynamic and pharmaco kinetic properties and therapeutic use in immuneregulatory disorders Drugs 1993;45: 953-1040.
2. Adams D, Davies G. Gingival hyperplasia associated with cyclosporine A. A report of two cases. Br Dent J 1984;157: 89-90.
3. Seymour RA, Elles JS, Thomason JM. Risk factors for drug-induced gingival over growth. J Clin Periodontol 2000;27: 217-223.
4. Chanders V, Chopra K. Effect of molsidomine and L-arginine in cyclosporine nephrotoxicity: role of nitric oxide. Toxicology 2005;207:463-474.
5. Klintman CBG, Iwazuki S, Starzi TE. Cyclosporine A hepatotoxicity in 66 renal allograft recipients. Transplantation 1981;32:488-489.
6. Laupacis A, Keown PA, Ulan RA, Sinclair NR, Stiller NR. Hyperbilirubinemia and cyclosporin A levels. Lancet 1981; 32:488-489.
7. Beaman M, Parvin S, Vertch PS, Wall J. Convulsions associated with cyclosporine A in renal transplant recipients. Br Med J 1985;290:139-140.
8. Starzi TE, Porter KA, Iwazuki S. Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporine-steroid therapy. Transplant Proc 1981;3:559-587.
9. Thiru S, Calne Y, Nagington J. Lymphoma in renal allograft patients treated with Cyclosporin A as one of the immunosuppressive agents. Transplant Proc 1981;3:359-364.
10. Guo J, Wang W, Yao L, Yan F. Local inflammation exacerbate cyclosporine A- induced gingival overgrowth in rats. Inflammation 2008;31:399-407.
11. The Canadian –European Randomized Control Trial group: Cyclosporin-induced remission of TDDM after early intervention: association of 1 yr of cyclosporin treatment with enhanced insulin secretion. Diabetes 1988;31:1574-1582
12. Petersen JS, Dysberg T, Karlsen AE, Molvig J, Michelsen B, Nerup J, Mandrup-Poulsen T. Canadian European Randomized Control Trial Group: Glutamic acid Decarboxylase (GAD 65) autoantibodies in prediction of beta cell function

and remission in recent onset IDDM after cyclosporin treatment. Diabetes 1994;43:1291-1296.
13. Eckard M, Schundler RA, Renner FC, Schief W, Padberg W, Weimer R, Bretzel RG, Brendel MD. New onset diabetes mellitus after renal transplantation. Transplant Proc 2009;41:2544-2545.
14. Gnatta D, Keitel E, Heineck I, Cardoso BD, Rodrigues AP, Michel K, Garcia VD. Use of tacrolimus and development of postransplant diabetes mellitus: A Brazilian single –center, observational study. Transplant Proc 2010; 42:475-478.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193:265-275.
16. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tennebaum SR. Analysis of nitrate, nitrite and [15 N] nitrate in biological fluids. Anal Biochem 1982;126: 131-138.
17. Walsh BJ, Thornton SC, Penny R, Breit SN. Microplate reader based quantitation of collagens. Anal Biochem 1992; 203:187-190.
18. Catanzaro OL, Dziubecki D, Calvo Lauria L, Martinez Ceron C, Rodriguez R R. Diabetes and its effects on dental pulp. J Oral Sci 2006;48:199-206.
19. Hefti AF, Eshenaur AE, Hassell TM, Stone C. Gingival overgrowth in cyclosporine A treated multiple sclerosis patients. J Periodontol 1994;65:744-749.
20. Chae HJ, Yun DH, Pae HO, Chung HT, Chase SW, Jung YK, Kim HR. Mechanism of cyclosporine –induced overgrowth in gingival. J Dent Res 2006;85:515-519.
21. Pasceri V, Cheng JS, Willerson JT, Yhe ET. Modulation of C-reactive protein mediated monocyte chemoattractant protein-1 induction in human endothelial cells by antiatheroesclerosis drugs. Circulation 2001;103:2531-2534.
22. Zimman B, Hanley AJ, Harris SB, Kwan J, Fantus IG. Circulating tumor necrosis factor –alpha concentrations in a native Canadian population with high rates of type 2 diabetes mellitus. J Clin Endocrinol Met 1999;84:272-278.
23. Pradham AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein interleukin 6, and risk of developing type 2 diabetes mellitus. J Am med Assoc 2001;286:327-334.
24. Kiname DF, Petersen M, Stathopoulou PG. Environmental and other modifying factors of the periodontal disease. Periodontal 2000;40:107-119.
25. Greenberg KV, Armitage GC, Shiboski CH. Gingival enlargement among renal transplant recipients in the era of new-generation immunosuppressants. J Periodontol 2008; 79:453-460.
26. Maldonado F, Tapia G, Ardiles L. Early hyperglycemia a risk factor for post-transplant diabetes mellitus among renal transplant recipients. Transplant Proc 2009;41:2664-2667.
27. Hassell TM, Hefti AF. Drug-induced gingival overgrowth: old problem, new problem. Crit Rev Oral Biol Med 1991; 2:103-137.
28. Nares S, Ng NC, Dill RE, Park B, Cutler CW, Iacopino AM. Cyclosporin A upregulates platelet-derived growth factor B chain in hyperplasic human gingigva J Periodontol 1996; 67:271-278.
29. Atilla G, Kutukculer N. Crevicular fluid interleukin-1 beta, tumor necrosis factor –alpha, and interleukin -6 levels in renal transplant patients receiving cyclosporine A J Periodontol 1998;69:784-790.
30. Hayland PL, Traynor PS, Myrillas T, Marly JJ, Linden GJ, Winter P, Leadbetter N, Cawston TE, Irwing CR. The effects of cyclosporin on the collagenolitic activity of gingival fibroblasts. J Periodontol 2003;74:437-445.
31. G urkan A, Emingil G, Oktem G, Selvi N, Afacan B, Tunc I, Toz H, Atilla G. Immunohistochemical analysis of nitric oxide synthase in cyclosprorin A-induced gingival overgrowth. J Periodontol 2009;80:1638-1647.
32. Yuan SY, Breslin JN, Perrin R, Gaudreault N, Guo M, Kargozaran H, Wu HM. Microvascular permeability in diabetes insulin resistance. Microcirculation 2007;14:563-573.
33. Sakallioglu EE, Luftfioglu M, Sakallioglu U, Diraman E, Keskiner I. Fluid dynamics of gingiva in diabetic and systematically healthy periodontitis patients. Arch Oral Biol 2008;53:646-651.