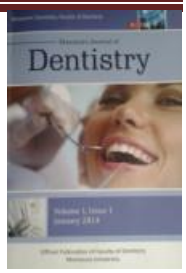
Effect of Cyclosporine A on Langerhans Cells in Albino Rat Gingiva



***Samar El Badry El Said*[1] , *Mohamed Abdel Rahman*[2] , *Yousry M. El Hawary*[3]**
[1]*Bachelor of dentistry, Mansoura University. Egypt.*
[2]*Assistant professor of Oral Biology, Faculty of Dentistry, Mansoura University. Egypt.*
[3]*Professor and head of Oral Biology department, Faculty of Dentistry, Mansoura University. Egypt.*

Abstract:
Objective: To detect the effect of Cyclosporine A (CsA) on langerhan's cells in rat gingiva (histologically and immunohistochemistry).
Methods: 45 male albino rats weighting 250-300 mg were used in this study and were divided into two groups, control group: consisted of 9 animals , were received olive oil for 4 week and experimental group: consisted of 36 animals were subdivided into three subgroups , each subgroup consisted of 12 animals were received daily CsA (10 , 20 & 30 mg/kg) by gastric tube. The drug administration stopped after four week. 4 animals of each experimental subgroup and 3 animals of control group were sacrified after 2 ,4 and 6 weeks successively . Gingival tissue were dissected and processed for histological (H&E) and immunohistochemical (S100) examination.
Results: In H&E results the control sections of the gingivae showed normal thickness of epithelium , keratin, normal rete pegs and normal arrangement of the four epithelial layers ,the experimental sections of the gingivae treated with CsA for 2 and 4 weeks showed increase in the thickness of epithelial layer, keratin layer with enlarged and elongated rete pegs and at 6 weeks duration, the experimental sections of the gingivae showed almost restoring of normal epithelium thickness with rete pegs. The immunolabeled cells in control groups showed mild reaction for S100 antibodies. The cells of experimental groups underwent progressive reduction in number of +ve reaction with increasing doses of CsA. After 4 weeks of the experiment the immuno +ve reaction with S100 antibodies appeared to be resolved to a level near to normal. At 6 weeks duration, a noticeable increase of +ve S100 antibodies could be seen in all subgroups.
Conclusions: CsA causes a dose dependent reduction in the number of dendritic LCs in the rat gingival epithelium. After four weeks of treatment with CsA (at all doses) LCs has been resolved with marked recovery to a level near normal when stoppage of drug occurred.

Keywords: *Cyclosporine A, Langerhans cells, S100 stain.*

Introduction

Cyclosporin A (CsA) is a potent immunosuppressive agent, which is classically used in organ transplantation to prevent allograft rejection [1] Indeed, CsA is known to reversibly block the early events in T cell as well as in B cell and natural killer cell responses leading to an alteration of the immune response [2]. Furthermore, CsA has been shown to impair the antigen (Ag)-presenting cell (APC) functions of several cell types, including dendritic cells (DC), Langerhans cells, and, monocytes [3].

LCs are localized to stratified epithelium of the epidermis and mucosa, and present antigens to T cells after engulfing the antigen and migrating to the lymphoid system [4].

LCs in sections stained with routine hematoxylin and eosin stains cannot be well appreciated. They appear as 'clear cells' having a clear halo around their nuclei [5].

The immunolabeling of LCs was planned with polyclonal antibodies to protein S100 . The protein S-100 has been identified in many cell types, such as Schwann cells, chondrocytes, Langerhans cells and other cells of neural origin. The expression of this protein was also observed in several types of neoplasias, especially of neural origin, salivary gland neoplasias, granular cell neoplasias, muscular neoplasias, gland neoplasias, granular cell neoplasias, muscular neoplasias, chondrosarcomas, in nearly 95% of melanomas, and also in Langerhans cells found in the Langerhans cell disease [6].

Materials and methods

Forty five male albino rats approximately 200-250 gm in weight were used in this study The animals were housed in individual cage, fed a standard diet and free access to water. The animals were divided into:

Group 1 (control group): consisted of 9 animals, received olive oil only for 4 weeks.

Group 2 (experimental group): consisted of 36 animals were subdivided into three subgroups:

- subgroup A : consisted of 12 animals , received daily cyclosporine A (10 mg/kg) by gastric tube.
- subgroup B: consisted of 12 animals , received daily cyclosporine A (20mg /kg) by gastric tube.
- Subgroup C : Consisted of 12 animals , received daily cyclosporine A (30 mg/kg) by gastric tube.

The drug administration was stopped after 4 weeks. 4 animals of each experimental subgroup and 3 animals of control group were sacrified by cervical dislocation after 2, 4 and 6 weeks successively. Gingival tissue were dissected and processed for histological (H&E) and immunohistochemical (S100) examination.

Statistical analysis

Data were tabulated, coded then analyzed using the computer program SPSS version 17.0

Analytical statistics

In the statistical comparison between the different groups was done by ANOVA (analysis of variance) to compare between more than two groups of numerical data followed by post-hoc tukey for multiple comparisons.

Results

In H&E results the control sections of the gingivae showed normal thickness of epithelium, keratin, normal rete pegs and normal arrangement of the four epithelial layers ,the experimental sections of the gingivae treated with CsA for 2 and 4 weeks showed increase in the thickness of epithelial layer, keratin layer with enlarged and elongated rete pegs and at 6 weeks duration, the experimental sections of the gingivae showed almost restoring of normal epithelium thickness with rete pegs. The immunolabeled cells in control groups appeared mildly in suprabasal nucleated cell layers. The cells of experimental groups underwent progressive reduction in number of +ve reaction with increasing doses of CsA. After 4 weeks of the experiment the immuno +ve reaction with S100 antibodies appeared to be resolved to a level near to normal. At 6 weeks duration, a noticeable increase of +ve S100 antibodies could be seen in all subgroups (Figs. 1,2,3).

Discussion

The action of CsA depends primarily on selective suppression of T cell activation by inhibiting the transcription of several cytokine genes [7]. A secondary mechanism of action proposed for CsA is an interference with the accessory function of antigen presenting cells, especially LCs [3].

In Hematoxylin and Eosin results the control sections of the gingivae showed normal thickness of epithelium, keratin, normal rete pegs and normal arrangement of the four epithelial layers with some degree of interdigitation between the epithelium and connective tissue. Each layer could be distinguished from others. Normal connective tissue separated from epithelium by basement membrane. These are in accordance with normal histological structure of gingival epithelial tissue that described by Garant [8].

In this study, the experimental sections of the gingivae treated with CsA for 2 and 4 weeks showed increase in the thickness of epithelial layer, keratin layer with enlarged and elongated rete pegs. This result was similar to previous researches contributing that the side effects of CsA depend upon factors include dosage and duration [9,10].

At 6 weeks duration, the experimental sections of the gingivae showed almost restoring of normal epithelium thickness with rete pegs. This agreed with the previous study done by Eric et al. [11] they said that CsA induces rapidly reversible mode of action.

In this study, the immunohistochemical results in control groups appeared mildly in suprabasal nucleated cell layers. This come in accordance with the studies of Bacci et al. [12] who made their studies on mice and Régnier et al. [13] who made their studies on human epidermis.

In our study, we found that, after 2 weeks of treatment with CsA in all doses, a dose dependent reduction occured in the number of dendritic LCs. These come in accordance with the data reported by Borghi et al. [14] who studied the effect of CsA on differentiation of LCs in the rat epidermis upon systemic treatment at different doses.

After 4 weeks of the experiment the immune +ve reaction with S100 antibodies appeared to be resolved to a level near to normal . These findings coincide with the results obtained by Horrocks et al. [15] who studied the differential effects of cyclosporine A on Langerhans cells and regulatory T-cell populations in severe psoriasis where, they found that, numbers of epidermal LCs increased substantially during resolution of the skin lesions.

At 6 weeks duration, a noticeable increase of +ve S100 antibodies could be seen in all subgroups . This agreed with the previous study done by Eric et al. [11] they said that CsA induces rapidly reversible mode of action. This reversible immunosuppression comes from the blocking of the transcription of genes encoding several cytokines, in particular interleukin (IL)-2. The molecular mode of action involves the specific binding of CsA to an intracellular protein, cyclophilin-1, and belonging to the immunophilin family. Then the cyclophilin–CsA complex thus formed inhibits calcineurin, which is an enzyme involved in the nuclear translocation of the cytoplasmic component of NF-AT, an essential transcription factor of the IL-2 gene [16]. The absence of IL-2 synthesis prevents the activation and the proliferation of T lymphocytes as well as B lymphocytes and Langerhans cells [17].

Conclusion

We should like to conclude that CsA causes a dose dependent reduction in the number of dendritic LCs in the rat gingival epithelium. After four weeks of treatment with CsA (at all doses) LCs has been resolved to a level near to normal. After two weeks from the stoppage of administration of CsA (in all subgroups), the numbers of LCs increased to be nearly resemble the controls. This is due to the reversible mode of action of CsA .

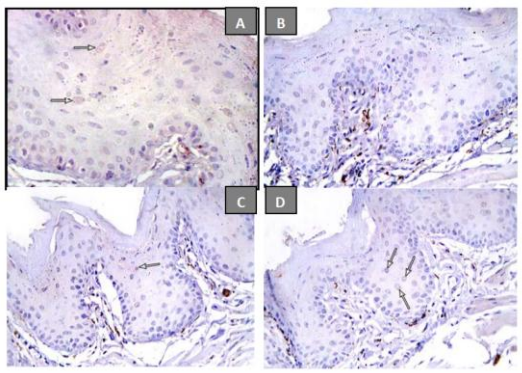


Figure 1: photomicrograph of S1oo immunostaining at the gingiva of a rat of (A) a control group showing little number of scattered expression of S100 antibody in parabasal and upper part of the section,(x-25o), (B) an experimental group (sub group A- 2 w) with no detection of any +ve antibody reaction for S100 antibody,(x -250), (C) an experimental group (sub group A- 4 w) with nearly one +ve reaction for S1oo antibody detected in the upper part of epithelium,(x -250), (D) an experimental group (sub group A- 6 w) with very little no of +ve cells can be seen hardly in the middle area of the section,(x -250).

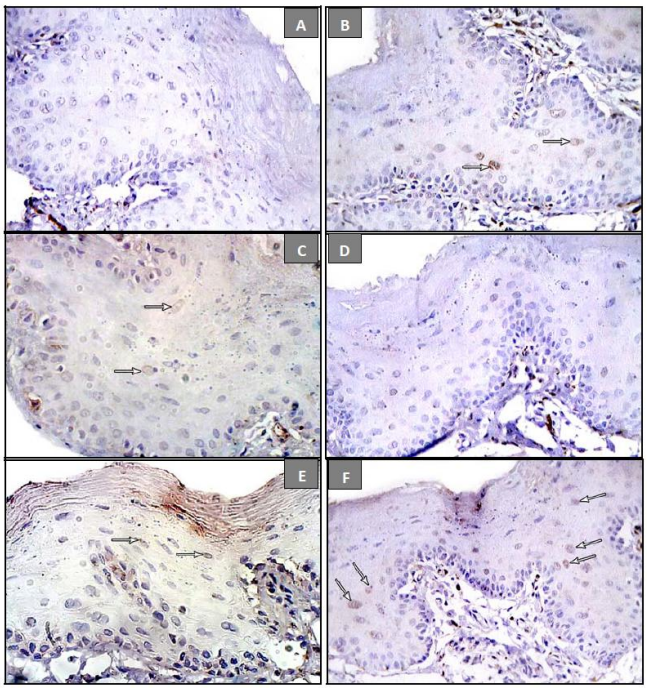


Figure.2: photomicrograph of S1oo immunostaining at the gingiva of a rat of (A) an experimental group (sub group B - 2 w) with no positive reaction for S1oo antibody in the section,(x – 250), (B) an experimental group (sub group B - 4 w) with little +ve reaction for S1oo antibody in the middle part of the section (arrows), (x -250), (C) an experimental group (sub group B - 6 w) showing little +ve antibody reaction in the parabasaland upper areas of the section (arrows).The immunoreactive cells located exclusively in the basal layer are probably melanocytes, (x-250), (D) an experimental group (sub group C- 2w) with no +ve reaction, (x-250), (E) an experimental group (sub group C- 4w) with little +ve reaction noted in the higher level of the section, (arrows), (x-250), (F) an experimental group (sub group C- 6w) showing increase in the no of +ve reaction distributed in the parabasal and middle area of the section, (arrows), (x -250).

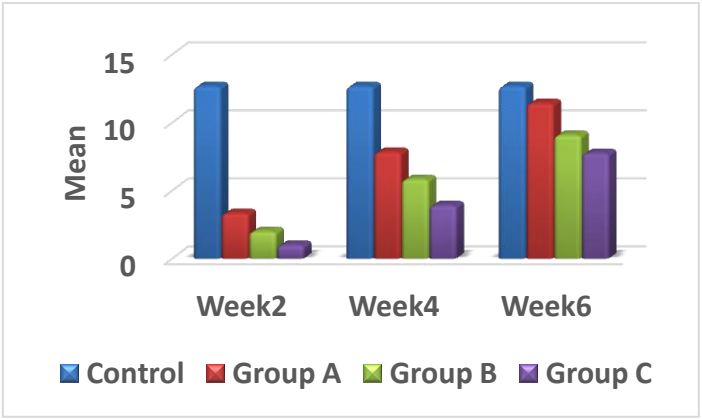


Figure.3: Chart diagram illustrating the difference in means of S100 expression in control group and different duration & concentration of CsA .

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