Macroscopic evaluation of the dimensional changes in the gingiva of sprague dawley rats administered sirolimus in different dosages over a period of sixteen weeks – *Role of MTOR inhibitors in causing gingival overgrowth*

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Abstract— The study was carried out to morphologically evaluate the effects and compare the magnitude of enlargement in the gingiva of rats, on administration of sirolimus, in three different dosages against the group administered a placebo. The experiment was performed on 5 week old, male Sprague Dawley rats, weighing 150 – 220 g.The animals were housed in pairs, in plastic bottomed cages, with husk as a bedding; in well ventilated rooms subjected to normal atmospheric conditions at 21°C and the same regimen of lighting (12 hours of light/ dark cycle) at the Central Animal House, Anamalai University and fed with a standard pellet diet and water ad libitum. After administration of the drugs, an impression was made of the rat mandibular incisal region, in polysiloxane impression material at the end of the IV, VIII and XII and XIV week and study models were made with die stone. A digital caliper (with a resolution of 0.01mm) was used to record the following parameters i.e vertical height, bucco-lingual width and mesio-distal width. ANOVA Repeated Measures Test was carried out for statistical analysis. Rats administered sirolimus in low and high doses showed a similar and greater change in dimensions compared to the control group, at the end of the IV week, in comparison to the baseline. ($P<0.001$).However the group administered the higher dosage of sirolimus showed a greater increase in the gingival dimensions, at the end of the XII and XVI week, this could be attributed to the consistent exposure and the cumulative effect of the high dosage of the drug, over the long duration, indicating that the unwanted effect of the drug could be cumulative and time dependent.

Index Terms— Dentist, Esthetics, Gingival enlargement, Immunosuppressants, mTOR inhibitors Sirolimus, Sprague dawley rats

1 INTRODUCTION

The immunosuppressive medicines along with several innovations for graft survival have increased the life expentancy of the geriatric population.[1] A dentist now encounters patients, who have undergone transplantation quite frequently, since periodontal health is usually compromised in these individuals.[2] Nephrotoxicity and Neurotoxicity are well recognized side effects of calcineurin inhibitors , like cyclosporine and tacrolimus, that have been documented, owing to which nearly 20% of liver transplant recipients experience chronic renal failure within 5 years[3].For patients with calcineurin inhibitor induced nephrotoxicity, conversion to sirolimus therapy has proved to be effective with ensuing improvements in renal function.[4,5] Sirolimus has been studied extensively in the past few years, for its beneficial effects over calcineurin inhibitors.[4,5,6,7,8,9] However the impact of sirolimus, on gingival health, has not been studied exclusively in transplantation patients and there is no conclusive literature on animal models like the Sprague dawley rats, evaluating the effects of the drug, from an oral perspective.[10] Existing literature on sirolimus, fails to evaluate the dosage and time related effects of the drug, with most studies being cross sectional in nature and findings confounded by the concomitant use of other drugs that cause gingival enlargement.[11]

Hence this study was undertaken to morphologically evaluate the effects of sirolimus, key component of the CNI-free regime, i.e; MTOR inhibitors, on the gingiva of Sprague dawley rats and to compare and study the magnitude of gingival overgrowth due to varying dosages of Sirolimus, over a period of 16 weeks.

MATERIALS AND METHODS

The experiments were performed on 5-week old male Sprague dawley rats, weighing 150 – 220 g. The animals were randomly distributed into 4 groups of 10 rats each. The animals were housed, in similar conditions, in plastic-bottomed cages, with access to food and water ad libitum. The cages were placed in well ventilated rooms, subjected to normal atmospheric conditions and 12 hours of light and dark cycles alternatively.

Ethical Approbation

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Rajah Muthiah Medical College, Annamalai University (Central Animal House Registration Number 160/1999/CPSEA). The National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) has been strictly followed in this study

Study Groups

Group 1: Rats were administered Sirolimus, 1.5 mg/kg body weight, in normal

saline (0.9% w/v) daily, for 16 weeks.

Group 2: Rats were administered Sirolimus, 2 mg/kg body weight in normal saline (0.9% w/v) daily, for 16 weeks.

Group 3: Rats were administered Sirolimus 3mg/kg body weight in normal saline(0.9%w/v) daily for 16 weeks.

Group 4: Control group - Rats were administered normal saline (0.9% w/v) alone daily, for 16 weeks.

fig 1: Making an Impression of the rat mandibular in cisal region
fig 2: Fabrication of the study model

IMPRESSION MAKING

An impression was made of the rat mandibular incisal region, in polysiloxane impression material.(fig1) A preliminary study model was made in die stone.(fig2) Acrylic trays of an approximate size, were then fabricated for all the rats, with the help of this study model.The same procedure was repeated at the end of the IV, VIII and XII and XVI week, using the pre-fabricated acrylic trays, with polysiloxane impression material, to evaluate the changes in the gingival dimensions, following the administration of the drugs.

RECORDING THE GINGIVAL DIMENSIONS FROM THE CAST

The dimension of the inter-dental gingiva and the keratinized gingiva was measured on the study cast using a digital caliper (with a resolution of 0.01mm) and the following parameters were recorded i.e, bucco-lingual width, mesio-distal width and the vertical height.(fig3)

fig 3. Recording the dimensions of the cast

STATISTICAL ANALYSIS

Statistical analysis was then carried out and simultaneous comparisons were made between the groups and within the groups, by using ANOVA Repeated Measures Test.

RESULTS Gingival enlargement, was apparent in the test groups at the end of the IV week itself and the extent of gingival overgrowth was progressive in all the test groups till the end of the XVI week.(fig4)(fig5)(fig6)(fig7) However the test group receiving 3mg/kg of the drug showed a greater percentage increase compared to the groups receiving lesser doses. The gingival enlargement in all three test groups was statistically significant as compared to the control group.

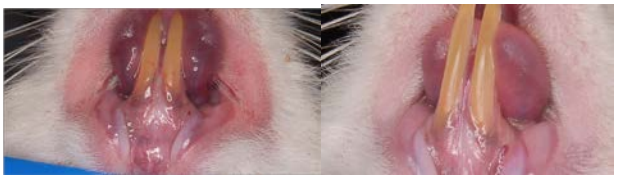

fig 4. Control group at 16 weeks

fig 5. Sirolimus 1.5mg/kg group at 16 weeks

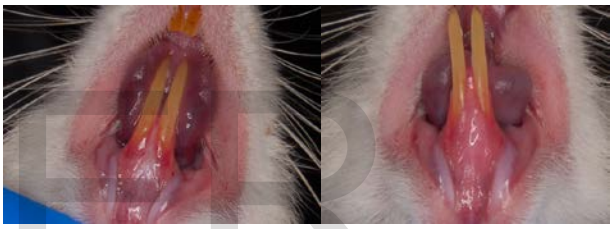

fig 6. Sirolimus 2 mg/kg group at 16 weeks

fig7.. Sirolimus 3 mg/kg at 16 weeks

The test group receiving the highest dosage of 3mg/kg Sirolimus, showed the maximum increase in gingival dimension at the end of the twelfth and sixteenth week, in all the measured dimensions, of the inter-dental papilla and the keratinized ginigva i.e vertical height, bucco-lingual width and mesio-distal width, in the study models, when recorded with a digital caliper (resolution capacity of 0.01 mm), (Table IA, IIA, IIIA, IVA, VA)

GROUP	BASELINE		IV WEEK		VIII WEEK		XII WEEK		XIV WEEK	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sirolimus 1.5g/kg (1)	3.23	0.01	3.29	0.01	3.37	0.02	3.42	0.01	4.06	0.01
Sirolimus 2mg/kg (2)	3.23	0.01	3.33	0.01	3.43	0.01	3.52	0.01	4.16	0.01
Sirolimus 3mg/kg (3)	3.23	0.01	3.53	0.01	4.08	0.01	5.12	0.01	6.65	0.01
Control (4)	3.23	0.01	3.27	0.01	3.35	0.01	3.42	0.01	4.04	0.01

IA: Comparison of the vertical height (mm) of the inter-dental papilla between the

study groups.

GROUP	BASELINE		IV WEEK		VIII WEEK		XII WEEK		XIV WEEK	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sirolimus 1.5g/kg (1)	6.93	0.01	7.22	0.01	7.28	0.01	7.36	0.01	7.68	0.01
Sirolimus 2mg/kg (2)	6.93	0.01	7.36	0.01	7.42	0.01	7.53	0.01	7.72	0.01
Sirolimus 3mg/kg (3)	6.94	0.01	7.56	0.01	8.11	0.01	9.15	0.01	10.68	0.01
Control (4)	6.93	0.01	7.21	0.01	7.27	0.01	7.35	0.01	7.67	0.01

IIA: Comparison of the vertical height (mm) of the keratinized gingiva between the study groups

GROUP	BASELINE		IV WEEK		VIII WEEK		XII WEEK		XI
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Me
Sirolimus 1.5g/kg (1)	2.18	0.01	2.21	0.01	2.53	0.01	2.62	0.01	2.7
Sirolimus 2mg/kg (2)	2.18	0.01	2.32	0.01	2.66	0.01	2.73	0.01	2.8
Sirolimus 3mg/kg (3)	2.18	0.01	2.41	0.03	3.01	0.01	3.90	0.01	4.50	0.01
Control (4)	2.18	0.01	2.20	0.01	2.52	0.01	2.61	0.01	2.71	0.01

IIIA: Comparison of the mesio-distal width of the inter-dental papilla

GROUP	BASELINE		IV WEEK		VIII WEEK		XII WEEK		XIV WEEK	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sirolimus 1.5g/kg (1)	6.24	0.01	6.43	0.01	7.18	0.01	7.27	0.01	7.45	0.01
Sirolimus 2mg/kg (2)	6.24	0.01	6.51	0.01	7.24	0.01	7.33	0.01	7.46	0.01
Sirolimus 3mg/kg (3)	6.23	0.01	7.01	0.01	8.18	0.01	9.27	0.01	9.7	
Control (4)	6.23	0.01	6.42	0.01	7.17	0.01	7.26	0.01	7.44	0.0

IVA: Comparison of the mesio-distal width (mm) of the keratinized gingiva between the groups.

GROUP	BASELINE		IV WEEK		VIII WEEK		XII WEEK		XIV WEEK	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sirolimus 1.5g/kg (1)	3.83	0.01	4.05	0.01	5.19	0.01	5.22	0.01	5.37	0.01
Sirolimus 2mg/kg (2)	3.83	0.01	4.10	0.01	5.20	0.01	5.27	0.01	5.57	0.01
Sirolimus 3mg/kg (3)	3.83	0.01	4.36	0.01	5.30	0.01	6.58	0.01	6.93	0.01
Control (4)	3.83	0.01	4.04	0.01	5.18	0.01	5.21	0.01	5.36	0.01

VA :Comparison of the bucco–lingual width (mm) of the inter-dental papilla between the study groups**.**

The role of local factors as an inflammatory component, should be considered, while studying the effects of the drug, However, since all the rats were housed and fed according to a uniform protocol, this confounding factor would be present in all groups and hence not evaluated in detail in this study

We observed that the gingival enlargement caused by sirolimus is due to an increased dose of the drug and is time dependent. Sirolimus seems to have a cumulative effect on the gingival tissues, over a long duration, when administered, but only in high doses.

DISCUSSION

Cyclosporin and tacrolimus have been proven to cause gingival enlargement, in both human and animal, invitro and invivo models, with its patho-physiology clearly outlined in literature.[1,2,3] Sirolimus induced gingival enlargement, has been reported in in literature, with inconclusive evidence. **Cota et al** in a cross- sectional study done on Brazilian renal transplant patients, found gingival overgrowth, in a considerable number of subjects under Sirolimus based immunosuppressive regimens, although the relationship was not clinically significant.[10] In a prevalence study, by **Cota et al**[11], gingival overgrowth in subjects under immunosuppressive regimens based on cyclosporin, tacrolimus and sirolimus was evaluated. They found that the prevalence of GO was 60% for Cyclosporine,28% for Tacrolimus and 15% for Sirolimus groups. While a recent experimental study attempts to study the effects of the three drugs, cyclosporine, tacrolimus and Sirolimus on Sprague dawley rats,it fails to shed light on the effects of different dosages of each drug, especially sirolimus[12]

This study was undertaken to study the dosage and time related effects of sirolimus on the animal model, Sprague dawley rat. The morphological

changes in the gingiva of the rats was evaluated, by means of 3 test groups, each group being administered 3 different doses of the drug sirolimus and a control group. The test groups showed significant but similar changes in the gingival dimensions at the end of the fourth week and eighth week compared to baseline and the control group. However at the end of the twelfth week and the sixteenth week, the test group administered 3mg/kg of sirolimus, showed a pronounced increase, while the other two test groups, showing no significant change from the sixth week onwards, till the end of the twelfth week.

Sirolimus, belongs to a different class of immunosuppressants i.e mTOR inhibitors and are advocated as substitutes to calcineurin inhibitors, like cyclosporine and tacrolimus to overcome serious side effects like nephrotoxicity caused by them. Although sirolimus did induce gingival enlargement in the gingiva of Sprague dawley rats. It was dose related and was shown to increase in intensity, only when administered in high dosages, over a prolonged period. The test groups administered low doses, failed to cause further enlargement, even after a duration of 16 weeks. Because this is an animal study, extrapolation of this data has its limitations. However it may be concluded that although Sirolimus seems to have a cumulative effect on the gingival tissues, over a long duration, when administered, but it occurs only at high concentration. Hence practical application of this drug would require dose tritration with a well planned dosing interval inorder to avoid the unwanted periodontal side- effects, from a dental perspective. The findings need to be confirmed and substantiated with further longitudinal studies in patients, undergoing mTOR inhibitor therapy.

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