Impact of Sirolimus Versus Cyclosporin A Immunosuppressive Drug in Dog's Alveolar Bone

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Abstract: Sirolimus is a modern immunosuppressive drug that has a novel mechanism of action as it improves the patients' condition receiving transplant. This study aimed to asses the effects of sirolimus Vs cyclosporin A (CsA) immunosuppressive drug on teeth's alveolar bone. Fifteen Mongrel dogs were used in this study. They were classified into three equal groups. The 1st group is considered as control. The 2nd and 3rd groups were subjected to cyclosporin A and sirolimus treatment protocol, respectively up to 45 days. The parameters involved were 1) body weight (BW), 2) biochemical markers of serum osteocalcin (OC) and alkaline phosphatase (APH) levels. 3) Densitometric analysis for the mandibular alveolar bone at canine area using dual energy X-ray absorptiometry. 4) All animals were euthanized, mandibles were dissected and specimens taken from the canine areas (canine and its supporting bone) and specimens were processed to examined the alveolar bone changes at the end of the experiment and 5) histomorphometric analysis using Masson's trichrome stain evaluated the width of periodontal ligament. Results obtained revealed a significant decreases of both body weight and alveolar bone mineral density. Meanwhile, there were significant increases of periodontal ligament width, serum OC and APH. We concluded that both sirolimus and CsA drugs have adverse effects on the alveolar bone quality. Also, the sirolimus produced the worst effects regarding of BW, BMD of teeth's alveolar bone, serum OC and APH levels with evidence of osteoporosis.
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Key wards: Alveolar bone; Osteoporosis; Bone mineral density; Sirolimus; Cyclosporin A.

1. Introduction

The chronic post-renal transplantation complication subsequent to the long-term immunosuppressant treatment is bone loss associated with osteoporosis [Campistol et al., 2005]. Osteoporosis is a clinically important problem affecting from 6 to 15% of all patients in the first year after transplantation [Shane and Epstien 2001] with incidence of spontaneous osteoporotic fractures as high as 25%-65% due to reduced bone quality [Patel et al., 2001] and avascular bone necrosis [Hamdy et al, 2005]. The organ transplantation rejection has been dramatically reduced and the survival rate of transplanted patients has been improved by using modern immunosuppressant such as sirolimus (Rapamune) [Casas-Melley et al., 2004]. Rapamune; (C51H79NO13, molecular weight 914.2) is a naturally occurring water-insoluble macrolide antibiotic produced by the *Streptomyces hygroscopicus* bacterium, which was serendipitously isolated by Dr. Suren Seghal from soil samples taken from the Vai Atare region of Easter Island [Sehgal 2003]. It was approved by the United States FDA in 1999, as an immunosuppressant to prevent allograft rejection in several phase 2 and phase 3 clinical trials either in combination with cyclosporin A (CsA) or used as a primary therapy [Johnson et al., 2001].

Other immunosuppressive drugs commonly used in transplantation, such as CsA and tacrolimus (FK506), have also been shown to have a deleterious effect on bone mineral metabolism in the rat [Schlosberg et al., 1989]. Unlike CsA or tacrolimus, sirolimus drug is structurally related to tacrolimus but functions through a different pathway; it has no effect on calcineurin phosphatase avoiding the long-term calcineurin inhibitor side effects. These side effects includes reduce glomerular filtration rate, nephrotoxicity, hypertension and diabetes mellitus therefore conferring a different safety profile [Campistol et al., 2005].

The sirolimus mechanism of action is different, where it blocks T-cell proliferation at a later stage than calcineurin inhibitors by affecting interleukin (IL)-2- and IL-4 induced signal transduction pathways rather than having a direct effect on cytokine production [Luo at al., 1993]. The inhibition of T-lymphocyte activation and proliferation that occurs in response to antigenic and cytokine lead to inhibits antibody production [Saunders et al., 2001]. In the cells, the macrocyclic immunomodulatory drug that are bioactive only when complexes with

ubiquitous intracellular binding proteins immunophilins and a specific effectors' protein to generate an immunosuppressive complex, immunophilin FK506-binding protein (FKBP)12; this complex binds to and inhibits the activation of the mammalian target of rapamune (mTOR) which inhibiting the progression from the G1 to the S phase of the cell cycle [Tsang et al., 2007]. mTOR is a 289 kDa phosphatidylinositol 3-kinase-related kinase which is evolutionarily conserved from yeast to mammals. It has a critical role in promoting cellular growth and differentiation, cell cycle progression, apoptosis and organ size [Carlson et al., 1993].

Moreover, sirolimus has a beneficial effect as it prevents the onset or ameliorates the evolution of various experimental autoimmune diseases, such as murine systemic lupus erythematosus, type I diabetes mellitus, collagen-induced arthritis, or adjuvant arthritis, experimental autoimmune uveoretinitis and experimental allergic encephalomyelitis in the rat,. The most notable side effects of sirolimus were hyperlipidemia (both hypercholesterolemia and hypertriglyceridemia) and thrombocytopenia and leukopenia, which may lower the lifespan of the transplanted patients [Deters et al., 2001]

There is a doubt whether the sirolimus had no effect on bone metabolism of supporting teeth structures while the concern has been raised over the unique structure of alveolar bone supporting teeth in patients received tissue transplant as it expressed extensive macroscopic and microscopic changes during occlusion and mastication. So; this study intended to assess the effect of sirolimus on alveolar bone and periodontal ligament supporting teeth structures versus different conventional immunosuppressant like CsA.

2. Materials and methods:

All experimental protocols involving animals conformed to procedures described in the Guiding Principles for the Use of Laboratory Animals according to the ethical committee for animal care (Laboratory Animal Center, Mansoura University, Egypt). The experimental study was conducted for 45 days. Fifteen adult healthy males, Mongrel dogs, aged between 8 and 10 months of age pathogenic free were selected for the present experimental work. Each dog was subjected to the same conditions through caging individually with proper light and temperature and feeding soft food under controlled conditions of humidity (50 ± 10%), light (12-h light/dark cycle), and temperature (23 ± 2° C). The dogs were divided into three equal groups of five dogs. In 1st group, the dogs did not receive any medication and were considered as a control. The animals of 2nd group; received CsA (Sandimmune, Novartis, E. Hannover, NJ, USA) which were given in a dose of 15 mg/kg/twice daily. Serum samples (taken after 1 week of start) for the determination of trough CsA levels were taken and stored at -20° C. CsA assay was performed using a monoclonal fluorescence polarization immunoassay (Abbott Laboratories, Abbott Park, IL USA). Trough levels were adjusted at the therapeutic range of 200–300 ng/ml. While the dogs of 3rd group received sirolimus (Rapamune, Wyeth, 5 Giralda Farms and Madison, NJ, USA), 1 mg/kg/day [Sorror et al., 2008].

Body weight, biochemical markers and bone densitometry:

At end of experiment, the animals' body weights (BW) were recorded in each group. The biochemical markers were evaluated for serum osteocalcin radioimmunoassay and serum quantitative assay for alkaline phosphatase as the animals were referred to veterinary surgeons for obtaining the blood samples under sterile conditions via marginal vein of ear. Serum osteocalcin (OC) samples were measured by a previously described technique [Delmas et al., 1993]. While the serum concentrations of alkaline phosphatase (APH) was determined by the DGKC method (Biozyme Laboratories Ltd., South Wales UK) and measured with a KEM-O-MAT 2 (Coulter Electronics Ltd, Florida, USA). The bone densitometry scans of the alveolar bone in canine area of the mandible were performed by using dual energy X-ray absorptiometry; (DEXA) (DXL, Calscan, Demetech AB, Stockholm, Sweden) to measure the bone mineral density in mg/cm2 with software for animals (Hologic, INC, Waltham, USA).

Histological and histomorphometric examination

At the end of experiment; each animal was sedated with an intramuscular injection of 1 mg/Kg Xylazine. General anesthesia was induced with an intramuscular injection of 6-mg/Kg Thiopentone. Before the beginning of all experimental procedures the trachea was intubated and general anesthesia was maintained using halothane (1.5–2.5%) in oxygen, delivered through a semi-closed breathing circuit. The mandibles of all groups were dissected and the specimens taken from the canine areas included the canine' tooth and its supporting bone from each side that were processed and prepared for Haematoxylin and Eosin staining to examine the alveolar bone and periodontal ligament tissue. In addition, the histomorphometric examination was performed to evaluate the width of the periodontal ligament by using Masson's trichrome stain. The measurements performed manually using a micrometer integrating eyepiece; the distances were analyzed including

sections within two fields at x250 magnification from cementum to alveolar bone trabeculae in different three point areas at cervical, middle and apical part by two different investigators.

Statistical analysis: The laboratory and biochemical parameters were analyzed with one way and two ways ANOVA using SPSS 17.0 for windows (SPSS Inc, Chicago, Illinois, USA). The data were analyzed for histomorphometric examination, biochemical markers like OC and APH levels, then the body weight of dogs and bone densitometry in mandibular' canine area of alveolar bone in each group, the data were expressed as mean and SD and the *P* value <0.05 was considered significant.

3. Results:

1) Body weight:

The means of BW were significantly decreased in CsA group and in sirolimus one at *P*<0.05 level Table(1).

2) Biochemical marker:

A significant increase of OC and APH serum levels in sirolimus and CsA groups compared to control one. The mean values for OC and APH were significantly increased in sirolimus group in comparison with CsA one at *P*<0.05 level [Table 1].

Table1: BW, BMD, OC and APH for the different groups

Group	BW (kg)	BMD (mg/cm2)	OC (ng/ml-1)	APH (U/l)
Control	8.87^{a} ±0.77	669.2^{a} ±71.54	46.71^{a} ±1.29	**12.28^{a} ± 0.48**
CsA	6.27^{b} ±0.54	453.2^{b} ±26.54	52.82^{b} ±2.54	**15.46^{b} ±1.24**
Sirolimus	3.45^{c}±0.158	338^{c} ±14.46	61.62^{c} ±2.88	**17.26^{c} ±1.12**
***F* Value**	238.408	140.587	103.15	**62.616**
***P* value**	**0.05**	**0.05**	**0.05**	**0.05**

Values with same superscripts are non significant

3) Bone densitometry:

ANOVA test for the BMD means revealed that the mean differences were significant between two groups at *P*<0.05 where the LSD multiple comparisons showed significant difference between each of two groups. The mean BMD was decreased in CsA group than in sirolimus one.

4) Histological finding:

The histological sections of the control group (Fig 1A) at the middle part of canine dog's tooth showed normal architectures of dentine formation (D), cementum (C), periodontal ligament (PD), bundle bone (BD) and supporting lamellated bone (SP). The CsA treated group (Fig 1B) sections showed multiple areas of bundle bone (BP) resorption with widened bone marrow spaces (MS) in a little area, the architecture of supporting alveolar bone (SP) seem to be normal. While the Sirolimus treated group section (Fig 1C) showed irregularly resorbed bundle bone (BP) with invasion of less vascular periodontal ligament (PD) in the resorption sites and marrow spaces. Loss of lamellated regular pattern of supporting alveolar bone (SP) and subsequent rarified osteones and bony trabeculae, with widened marrow spaces can be seen.

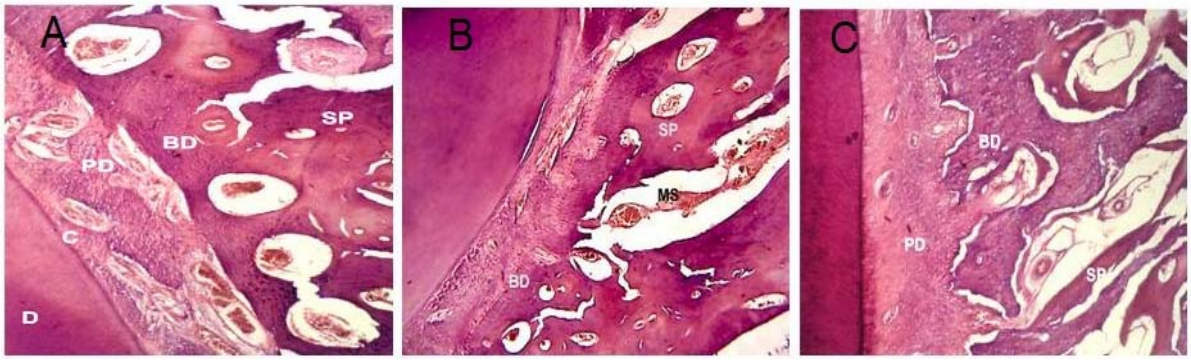

Fig 1: photomicrograph at the middle part of canine dog's tooth of the control group (A), CsA treated group (B) and sirolimus treated group (C) (H&E x400).

5) Histomorphometric result:

In the table [2]; there was a significant difference in thickness of periodontal ligament between the control Vs sirolimus and CsA group at level of $P<0.05$. There was no significant difference between the thickness of periodontal ligament of sirolimus and cyclosporin groups**.**

Table 2: Thickness of periodontal ligament at different locations for different groups.

Group	Thickness	Location
	Mean ±SD	Mean ±SD
Control	0.44^{a} ±0.10	0.96^{a} ±0.85
CsA	0.59^{b} ±0.10	1.00^{a} ±0.83
Sirolimus	0.64^{c} ±0.12	1.06^{a} ±0.82
F value	31.507	0.111
P value	0.05	0.895

Values with same superscripts are non significant

4. Discussion:

Sirolimus has emerged as one of the most promising immunosuppressive agents when used alone or in combination. The use of sirolimus is attractive as primary prophylaxis for chronic graft against host prevention not seen with other classes of immunosuppressant due to its unique properties that can offer several advantages [Cutler and Antin 2010] as a new approach to overcome the problem of chronic calcineurin inhibitor nephrotoxicity without increasing the risk of allograft rejection.

The experimental models like animal, in vitro were performed to investigate the effect of any materials by using any measures, the results from animal models may transfer to humans [Krocker et al., 2006] in addition to the microscopic finding which give more convenient tool for histological finding. In the present study, we selected the dogs as animals that have the ability to withstand the long term immunosuppressant drugs to evaluate the adverse effect on bone composition through biochemical and histological examination.

The result of present study showed evidence of osteoporosis in CsA and sirolimus group in comparing to control one appeared as significant increases of biochemical bone marker for OC and APH serum levels associated with significant decreases of BW of the animal and BMD at canine areas of alveolar bone in addition to the histological finding represented as resorption of bundle bone layer even exposing the lamellated bone with widening of bone marrow and significant increases of periodontal ligament width. The sirolimus group result was the worst.

In relation to CsA, results were in agreement with Yeoa, et al., 2007; who reported that the administration of CsA to rats at doses (15 mg/kg) showed significant bone resorption and trabecular bone loss, resulting in severe high turnover osteopenia and decrease trabecular bone volume. Consistent with the work by Arzate et al., 2005 that revealed an inhibition of osteoblast differentiation by CsA at high concentrations to mice and significant decreases of trabecular bone volume. Furthermore, non-cephalic whole body bone mineral density measured by DEXA was significantly decreased after treatment with high doses of CsA [Arzate et al., 2005 & Yeoa, et al., 2007].

Previous investigations about the effect of CsA on bone are in agreement with the current study that the histological observation showed increased bone remodeling with resorption exceeding formation in experimental animals. In contrast, the in vitro study revealed that CsA inhibits stimulated bone resorption and both osteoblast and osteoclast activity and inhibits the proliferation, number, mitogenesis, attachment and APH concentrations of osteoblasts [McCauley et al., 1992], but in vivo it apparently yielded increases bone turnover, increased bone formation with excessive resorption [Derfus et al., 1991]. There is evidence for both decreased bone formation and enhanced bone formation with excessive resorption could be attributed to differences in the particular bones investigated or in the microanatomy if the chosen animals were of different ages apparently contradictory patterns of bone turnover [Klein, 1981].

The result of APH and OC serum had significant increases in CsA group, this results were in agreement with the previous studies on humans and animals. In human renal transplant patients treated with CsA; it has greater serum concentrations of bone markers, APH and OC, which revealed an increased osteoblastic activity [Withold et al., 1994 & Bonnin et al., 1997]. These patients had firstly a decreased in serum APH that was noted one week after CsA administration; then; the enzyme returned to normal one month after administration and then increased significantly after 3-6 months Also; changes in the concentration of OC, showed a decreasing for a short time and then increasing, in renal transplant patients who received CsA [Bonnin et al., 1997]. Similarly, in the rat model, there was a tendency for APH to decrease 1–2 weeks after CsA administration and then return to normal at 4 weeks [McCauley et al., 1992].

The results of sirolimus treated group in present study showed significant decreases in BW that were in agreement with the study carried out by Sanchez and He, (2009) and their investigators; who reported that bone length decreased in young rats with normal

renal function treated with sirolimus at 2 mg/kg daily for 14 days accompanied by alterations in growth plate architecture; even it lowered the chondrocyte proliferation [Earl et al., 2001]; that in turn reflected on our opinion on body weight. The changes in trabecular bone modeling and remodeling with decrease in body length have been demonstrated in 10 week old rats after 2weeks of rapamune and they attributed that due to the anti-proliferative effects rapamune therapy which may have adverse effects on linear growth in young children [Sanchez and He, 2009].

This result was in contradiction with Joffe and coworkers (1993) as they revealed a transiently lowered serum OC levels without any affect on the trabecular bone volume or bone formation rate with higher dose of rapamycin at 2.5 mg/kg per day for 14 days, also; the in-vitro study demonstrated that the rapamune with high dose inhibits the osteoclast function, lessens bone resorption, decreases osteoblast proliferation and enhances osteoblast differentiation [Singha et al., 2008]. On the other hand; the in vivo study showed that the high doses of rapamune increase bone turnover reflected by the elevation of OC and non collagenous bone protein secreted by osteoblasts [Holstein et al., 2008]. Alvarez-Garcia et al.,(2007) demonstrated that rapamune reduces chondrocyte proliferation and maturation during endochondral ossification in growth plates of young rats which was associated with a decreased resorption of cartilage and an alteration of vascular invasion, they attributed this effect to a decreased vascular endothelial growth factor expression in hypertrophic chondrocytes after rapamune treatment. Also, rapamune had tested in early fracture healing at 2nd week post fracture by Holstein et al., (2008) and found that the treatment leads to a severe alteration, as indicated by a decreased torsional stiffness. This effect was associated with a significant inhibition of hard callus formation together with a dramatically reduced formation of new woven bone.

Conclusion: Within the limitation of the current study; both sirolimus and CsA drugs have adverse effects on the alveolar bone quality. The sirolimus produced the worst effects regarding of BW, BMD of teeth's alveolar bone, serum OC and APH levels with evidence of osteoporosis that reflected histologically as resorption of bundle layer of alveolar bone and exposure of lamellated supporting bone with subsequent increases of periodontal ligament width.

Recommendations: More researches must be done to examine new therapy or dietary supplement given to the transplant recipient patient suffering from long term immunosuppressant therapy to minimize and treat or at least to improve this complication and promote bone formation metabolism.

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Conflicts of interest: None.

References:

1. Alvarez-Garcia O; Carbajo-Perez E; Garcia E; Gil H; Molinos I; Rodriguez J, (2007): Rapamycin retards growth and causes marked alterations in the growth plate of young rats. Pediatr Nephro. l 7: 954–961.
2. Arzate H; Alvarez M. A. and Narayanan A. S. (2005): Cyclosporin A promotes mineralization by human cementoblastoma derived cells in culture, J. Periodontal. Res 40:218-224.
3. Bonnin M.R; Gonzalez M. T; Grino J. M; Cruzado J. M; Bover J; Martinez J.M. and Navarro M. A. (1997): Changes in serum osteocalcin levels in the follow-up of kidney transplantation. Ann. Clin. Biochem. 34: 651–655.
4. Campistol J.M; Holt D.W; Epstein S; Gioud-Paquet M; Rutault K; and Burke J.T. (2005): Bone metabolism in renal transplant patients treated with cyclosporine or sirolimus. Transpl Int.18:1028-1035.
5. Carlson R.P; Baeder, W.L; Caccese R.G; Warner L.M and Sehgal S.N (1993): Effects of orally administered rapamycin in animal models of arthritis and other autoimmune diseases. Ann. NY Acad. Sci. 685: 86–113.
6. Casas-Melley A.T; Falkenstein K.P; Flynn L.M; Ziegler V.L. and Dunn S.P. (2004): Improvement in renal function and rejection control in pediatric liver transplant recipients with the introduction of sirolimus. Pediatr Transplant 8: 362-366.
7. Cutler C. and Antin J.H. (2010): Sirolimus immunosuppression for graft-versus-host disease prophylaxis and therapy: an update. Current Opinion in Hematology 17: 500–504.
8. Delmas P.O; Wahner H.W; Mann K.G. and Riggs B.L. (1993): Assessment of bone turn-Over in postnaenopausal osteoporosis by measurement of serum bone gla-protein. Journal of Laboratory and Clinical Medicine 102: 470-6.

9. Derfus BA; Csuka M.E; Carrera G.F; Hanson G. A. and Smith R.E. (1991): Bone remodelling after growth factor and cyclosporine A therapy for aplastic anemia. J. Rheumatol. 18: 738–742.
10. Deters M; Nolte K; Kirchner G; Resch K and Kaever V (2001): Comparative Study Analyzing Effects of Sirolimus–Cyclosporin and Sirolimus–Tacrolimus Combinations on Bile Flow in the Rat. Digestive Diseases and Sciences 46: 2120-2126.
11. Earl Fu; Yao Dung Hsieh; Tai-Kum Mao and Chin Shen E (2001): A histomorphological investigation of the effect of cyclosporin on trabecular bone of the rat mandibular condyle. Archives of Oral Biology 46:1105-1110.
12. Hamdy A.F; El-agroudy A.E; Bakr M.A; Mostafa A; El-Baz M; El-Shahawy E. and Ghoneimm M.A.(2005): Comparison of sirolimus and low-dose tacrolimus versus sirolimus-based calcineurine inhibitor-free regimen in live donor renal transplantation. American J Transplantation 5:2531-2538.
13. Holstein J.H; Klein M; Garcia P; Histing T; Culemann U; Pizanis A; Laschke MW; Scheuer C; Meier C; Schorr H; Pohlemann T and Menger M.D. (2008): Rapamycin affects early fracture healing in mice. British Journal of Pharmacology 154:1055–1062.
14. Johnson R.W.G; Kreis H; Oberbauer R; Brattstrom C; Claesson K. and Eris J (2001): Sirolimus allows early cyclosporine withdrawal in renal transplantation resulting in improved renal function and lower blood pressure. Transplantation 72: 777-786.
15. Joffe I; Katz I; Sehgal S; Bex F; Kharode Y; Tamasi J and Epstein S (1993): Lack of change of cancellous bone volume with short-term use of the new immunosuppressant rapamycin in rats. Calcif Tis Int 53:45-52.
16. Klein L (1981): Steady-state relationship of calcium-45 between bone and blood: differences in growing dogs, chicks, and rats. Science 214:190–193.
17. Krocker D; Perka C; Tuischer J; Funk J; Tohtz S; Buttgereit F and Matziolis G. (2006): Effects of tacrolimus, cyclosporin A and sirolimus on MG63 cells. Transpl Int. 19:563-9.
18. Luo H;.Chen,H; Daloze P; St-Louis G. and Wu J. (1993): Anti-CD28 antibody- and IL-4-induced human T cell proliferation is sensitive to rapamycin. Clin Exp Immunol. 94: 371–376.
19. McCauley L.K; Rosol T. J. and Capen C. C. (1992): Effects of cyclosporin A on rat osteoblasts (ROS 17/2.8 cells) in vitro. Calcif. Tissue Int. 51: 291–297.
20. Patel S; Kwan J.T; McCloskey E; McGee G; Thomas G; Johnson D; Wills R; Ogunremi L. and Barron J (2001): Prevalence and causes of low bone density and fractures in kidney transplant patients. J Bone Miner Res. 16:1863-1870.
21. Saunders R.N; Metcalfe M.S. and Nicholson M.L. (2001): Rapamycin in transplantation: a review of evidence. Kidney Int. 59: 3.
22. Sanchez C.P. and He Y.Z. (2009): Bone growth during rapamycin therapy in young rats. BMC Pediatrics 9:3.
23. Schlosberg M; Movsowitz C; Epstein S; Ismail F; Fallon M.D. and Thomas S. (1989): The effect of cyclosporin A administration and its withdrawal on bone mineral metabolism in the rat. Endocrinology 124: 2179-2184.
24. Sehgal S.N (2003):. Sirolimus: Its discovery, biological properties, and mechanism of action, Transplant Proc. 35: 7–14.
25. Shane E. and Epstein S. (2001): Transplantation osteoporosis. Transplant Rev. 15:11-32.
26. Singha U; Jiang Y; Yu S; Luo M; Lu Y; Zhang J and Xiao G. (2008): Rapamycin inhibits osteoblast proliferation and differentiation in MC3T3-E1 cells and primary mouse bone marrow stromal cells. J Cell Biochem. 103:434-446.
27. Sorror M. L; Leisenring W; Mielcarek M; Baron F; Diaconescu R; Hogan W. J; Graves S.S and Storb R. (2008): Intensified postgrafting immunosuppression failed to assure long term engraftment of dog leukocyte antigen-identical canine marrow grafts after 1 gray total body irradiation. Transplan. 85:1023-1029.
28. Tsang C.K; Qi H. Liu L.F and Zheng X.F. (2007): Targeting mammalian target of rapamycin (mTOR) for health and diseases. Drug Discov Today 12:112-24.
29. Withold W; Degenhardt S; Castelli D; Heins M. and Grabensee (1994): Monitoring of osteoblast activity with an immunoradiometric assay for determination of bone alkaline phosphatase mass concentration in patients receiving renal transplants. Clin. Chim. Acta. 225: 137–146.
30. Yeo H; Beck L.H; McDonald J.M. and Zayzafoon M. (2007): Cyclosporin A elicits dose-dependent biphasic effects on osteoblast differentiation and bone formation. Bone 40:1502-1516.

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