Original Article

Bone Sparing Effects of Bisphosphonates in Cyclosporine-induced Alveolar Bone Loss: An Animal Study

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INTRODUCTION

The use of cyclosporine-A (Cs-A) as an immunosuppressant has revolutionized organ transplantation, which has become the management of choice for many patients. However, a well-documented adverse effect of this immunosuppressive agent is reported to be the drug-induced gingival overgrowth (DIGO).[1] There are convincing evidences showing that alveolar bone loss is an important negative side effect of this drug.[2] Bisphosphonates (BPs) are drugs that are chemical analogs of inorganic pyrophosphates and modulate host response.[3-5] BPs can be divided into two classes: nitrogen containing and non-nitrogen-containing BPs.[6,7] Risedronate sodium (RSN) is an amino BP, which, when taken up by the bone, acts as an anti-osteolytic agent. Risedronate is shown to be successful in preventing bone resorption in experimental periodontitis.[8] To date, various studies have studied the effects of Cs-A and BPs on alveolar bone hemostasis of increased osteoclasia and reduced bone formation at periodontal sites after Cs-A therapy. D’Angelo et al.[9] found BPs as an effective drug in the treatment of osteoporosis in renal transplant patients. Although there are inadequate data, the at hand study was undertaken with the aim of evaluating the effectiveness of simultaneous risedronate therapy on the Cs-induced alveolar bone loss in a rat model.

SUBJECTS AND METHODS

The experimental protocol was conducted in accordance with local guidelines on the welfare of experimental animals and with the approval of the Committee of Ethics in Animal Research of JMJ Medical College and Bapuji Dental College and Hospital. Forty adult male Wistar rats aged 6–8 weeks old were housed in temperature-controlled...
rooms. The rats were at random divided into four groups: Group I was the control group and injected normal saline daily. Group II received subcutaneous injection of Cs-A, 10mg/kg body weight daily; Group III received subcutaneous injection of risedronate, 0.3mg/kg body weight. Group IV received subcutaneous injection of both Cs-A and risedronate. The study was conducted for a period of 60 days. Blood samples were centrifuged and the serum samples were immediately stored at –20°C. The dissected jaws were fixed in 10% formalin.

To measure bone loss, images were captured using a 3-chip charge-coupled device (CCD) camera attached to a stereomicroscope with 5× objective. The distance from cusp tip to the alveolar bone crest in the mandibular first molar was measured at ×40 magnification. All measurements were made on the buccal alveolar process. Levels of osteocalcin (OC) in serum samples were analyzed by enzyme-linked immunosorbent assay (ELISA), carried out according to manufacturer’s recommendations. The serum calcium levels were measured using the O-cresolphthalein complexone colorimetric method using o-Cresolphthalein complexone as the substrate.

### Statistical analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA) for comparison between the four groups followed by Tukey’s post hoc tests for pairwise comparison. The values of \( P < 0.05 \) were considered statistically significant.

### Results

#### Alveolar bone loss

Measurement of alveolar bone loss in the mandibular molar teeth revealed significantly higher bone loss in the Cs-A and combination (Cs-A + RSN) groups in comparison to the saline control group (\( P < 0.05 \)). The alveolar bone loss was significantly lower in the combination group than the Cs-A-treated group (\( P < 0.05 \)) [Table 1].

### Calcium levels in serum

The serum calcium levels in the Cs-A group were significantly lower when compared to the saline control, risedronate, and the combination groups (\( P < 0.05 \)) [Table 2].

### Osteocalcin levels in serum

The highest serum OC levels were obtained in the combination group and the lowest in the Cs-A group [Table 3].

### Discussion

The use of Cs-A to prevent rejection of allograft tissue has stirred the forte of transplantation biology. Cs-A-induced gingival alterations have been observed in clinical cases and in animal models. Nonetheless, significance of Cs-A on alveolar bone has been focused upon recently and its action on long bones and alveolar bone has been explored. In this study, we evaluated the outcome of BP therapy in the prevention of alveolar bone loss associated with Cs-A in a well-characterized animal model. The result of the present investigation indicated that simultaneous risedronate therapy counteracted the bone resorption induced by Cs-A, but it did not have any effect on bone when used alone.

Previous experimental studies have shown that administration of Cs-A in immunosuppressive doses (10mg/kg body weight) for 60 days results in alveolar bone loss around the lower molars.\(^{[2,10]}\) In the normal

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**Table 1: Intergroup comparison of alveolar bone loss**

| Bone loss (µ) | Mean | SD | \( F^* \) Value, sig | Mean difference, sig** |
|--------------|------|----|----------------------|------------------------|
| Groups       | Mean | SD |                      | Group I | Group II | Group III | Group IV |
| Group I      | 58.25| 4.92| 17.7, \( P = 0.001 \) HS | –       | 11.56 S  | 2.7 NS    | 5.4 S    |
| Group II     | 67.85| 2.24| –                    | –       | –        | 8.9 S     | 6.1 S    |
| Group III    | 61.30| 3.65| –                    | –       | –        | –         | 2.7 NS   |
| Group IV     | 64.04| 3.57| –                    | –       | –        | –         | –        |

HS = highly significant, NS = not significant, S = significant, SD = standard deviation

*One-way analysis of variance (ANOVA) test, ** Tukey's post hoc test, \( P < 0.005 \)—significant

**Table 2: Intergroup comparison of serum calcium levels**

| Calcium (mg/dL) | Mean | SD | \( F^* \) Value, sig | Mean difference, sig** |
|-----------------|------|----|----------------------|------------------------|
| Groups          | Mean | SD |                      | Group I | Group II | Group III | Group IV |
| Group I         | 9.28 | 0.94| 20.9, \( P < 0.001 \) HS | –       | 6.08 S  | 0.15 NS   | 0.34 NS  |
| Group II        | 3.19 | 0.61| –                    | –       | –        | 5.9 S     | 5.7 S    |
| Group III       | 9.13 | 1.24| –                    | –       | –        | –         | 0.19 NS  |
| Group IV        | 8.94 | 3.73| –                    | –       | –        | –         | –        |

HS = highly significant, NS = not significant, S = significant, SD = standard deviation

*One-way analysis of variance (ANOVA) test, **Tukey's post hoc test
physiological situation, both bone formation and resorption progress in an evenhanded, regulated manner with osteoclastic bone resorption foregoing new bone formation by osteoblasts. It is believed that Cs-A therapy can bring about an imbalance in the dynamic bone remodeling cycle, with excess resorption far exceeding formation, leading to eventual loss of bone.\cite{11} Cs-A exerts its osteopenic effect via the T cell rather than directly on bone,\cite{12} by interfering in the cytokine activity on both osteoclasts and osteoblasts,\cite{13,14} thus influencing bone remodeling.

It is well acknowledged that prostaglandin of the E series can affect both the active mature osteoclasts as well as their precursors and are dominant mediators of osteoclastic bone resorption. Cs-A can also enhance release of arachidonic acid metabolites prostaglandin \(E_2\) (PG-\(E_2\)), which is a cyclooxygenase-2 metabolite and mediator of osteoclastic bone resorption.\cite{11} PGE\(_2\) formation induced by Cs-A is not due to an enhanced level or activity of the cyclooxygenase enzyme but rather to its action on the production of the metabolites of the lipoxygenase pathway, which can also stimulate osteoclastic bone resorption. Cs-A treatment resulted in increased concentrations of interleukin-1 \(\beta\) and inducible nitrous oxide (iNOS) messenger RNA (mRNA) expression in the absence of inflammation.\cite{14} These cytokines are considered to play a role in bone resorption, probably through the stimulation of osteoclast proliferation and differentiation, leading to bone loss.

BPs are a special class of drugs, which are very widely used in the field of medicine. The ability of BPs to inhibit bone resorption is well described in the literature. This effect has impelled their use in postmenopausal osteoporosis, corticosteroid-induced osteoporosis, and bone metastasis.\cite{7} Although their mechanism of action has not been fully elucidated, at the tissue level, BPs reduce bone turnover, which slows down total bone loss. At the cellular level, the target is inhibition of osteoclast recruitment, adhesion, shortening of lifespan of the osteoclasts, and inhibition of osteoclast activity either through a direct action or by action on cells that modulate osteoclast activity. Hence, in recent years, BPs have become powerful agents in combating bone resorption due to their anti-osteoclastic activity particularly the amino BPs such as risedronate. BP therapy has been proven to be effectual in bone loss reduction around dental implants in estrogen deficiency signifying the therapeutic benefit of the drug on bone metabolism.

The serum biomarkers of bone turnover correlated well with the other data in this study. Biochemical and morphometric analysis data presented in our study suggest that risedronate counterbalanced the Cs-A-induced alveolar bone loss. OC, which is mostly produced by osteoblasts, is a valid marker of osteoblast activity. It could be regarded as a precise indicator of the function of cells responsible for bone formation and remodeling. According to our present findings, the combined administration of risedronate and Cs-A resulted in significantly higher serum OC levels than all the other groups and Cs-A group resulted in a significantly lower serum OC levels suggesting that Risedronate counteracts the negative effects of Cs-A on alveolar bone.

A marked decrease in serum calcium level in the Cs-A-treated group was statistically significant when compared to the other groups observed in our study. These findings are in concordance with previous studies,\cite{10} which suggested that the decreased levels of serum calcium can be attributed to the non-specific effect of Cs-A due to increased excretion by kidney and/or increased bone uptake.

**Conclusion**

As such, and within the limitations of this study, we conclude that alveolar bone loss is a possible side effect of Cs-A and treatment with risedronate is a beneficial therapeutic option that favors the normalization of Cs-A-induced alterations in bone metabolism. It modulates the host response to the action of Cs-A on the inflammatory cells and cytokines, which mediate alveolar bone resorption having no negative effects on the alveolar bone in the weekly dosage of 0.3 mg/kg body weight when injected subcutaneously. Additional studies should be designed and conducted in order to

| Osteocalcin (ng/mL) | Mean difference, sig** |
|---------------------|------------------------|
| **Groups**          | **Group I**            | **Group II**          | **Group III**          | **Group IV**          |
| Group I             | 67.68 ± 5.43           | –                     | 9.7 S                  | 1.7 NS                | 13.5 S                |
| Group II            | 57.95 ± 5.06           | –                     | –                      | 11.4 S                | 23.3 S                |
| Group III           | 69.42 ± 5.57           | –                     | –                      | –                     | 11.8 S                |
| Group IV            | 81.26 ± 5.97           | –                     | –                      | –                     | –                     |

HS = highly significant, NS = not significant, S = significant, SD = standard deviation

*One-way analysis of variance (ANOVA) test, **Tukey’s post hoc test
facilitate understanding of the biological mechanisms for the differential actions of Cs-A and risedronate at the molecular levels, which still remain ambiguous.

**Financial support and sponsorship**
Nil.

**Conflicts of interest**
There are no conflicts of interest.

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