Dental Alveolar Bony Alterations Induced by Ovariectomy or Cyclosporine A in Rats

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Abstract

PURPOSE: Because of the insufficient knowledge of osteoporosis in oral and dental skeletons, the purpose of this study was to investigate the dental alveolar bony alterations after ovariectomy or administration of cyclosporine A （CsA） in rats.

METHODS: Twenty-seven female SD rats were designed into control, ovariectomy and CsA-treated groups. All the animals received maxillary molars extractions. The nine rats of ovariectomy group then had surgical ovariectomy operation. After three weeks of healing, CsA-treated rats received daily CsA （15 mg/kg）, whereas the control and ovariectomy rats had mineral oil for 6 weeks. Three animals per group were killed at the end of week 2, 4 and 6. At one day and seven days before the sacrificing, the bony markers （calcium and alizarin red） were injected. Right and left maxillary ridges were prepared by undecalcified and decalcified histological procedures for the examination of bone formation （bony markers labeling） and bone resorption （Tartrate-resistant acid phosphatase, TRAP, staining）, respectively. The mandible ridges were fixed and prepared for scanning electron microscopy （SEM）.

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收稿日期：2004.11.30 修改日期：2005.4.28 接受日期：2005.5.11

RESULTS: In mandible body, a pattern of central trabecular bone surrounded with condensed cortex was observed in control animals with SEM; however, the loss of bone and fine trabecular bones were observed in rats received OVX and CsA. In the control group, the mandible tooth ridge zone, a dense bony pattern with scattered small marrow spaces was found in the inter-molar ridge, and a peripheral cortex with the central trabecular bone was noted in the intra-molar ridge. In OVX and CsA groups, the trabecular bone showed less and thin, especially in the inter-molar ridge. The maxillary edentulous ridge was examined only at week-4. Decreased alveolar bone volume, but increased bone-specific surface, was found in both OVX and CsA groups. Bony deposition between two fluorescent lines was less in OVX or CSA group if compared with that in control group; however, no difference of the bony resorption by TRAP staining was shown among three examined groups.

SUGGESTIONS: After OVX or CsA therapy, a pattern of loss and fine trabecular bone in mandible and a less bony deposition in maxillary edentulous ridges were observed if compared with the control therapy. Due to the limitation of study, we suggest that （i） an osteopinea/osteoporosis in the oral skeleton can be induced by OVX or CsA（ii）an inhibitory effect on the oral dental bone formation might be associated with the bony alterations.

Key Words：Cyclosporine A、Dental alveolus、Osteoporosis、Ovariectomy

卵巢切除或環孢靈藥物對大白鼠齒槽骨變化之研究

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摘　要

目的：由於骨質疏鬆危險因子之知識缺乏，因此本研究之目的在比較卵巢切除術或環孢靈藥物對大白鼠齒槽骨之變化。

方法：本實驗共選取 27 隻 S-D 雌性大白鼠，分成 3 組。在全身麻醉下摘除上顎臼齒，並將 9 隻白鼠逕行卵巢切除術（卵巢切除組）。經三週之癒合後，環孢靈組動物開始接受每公斤 15 毫克之餵藥，其餘控制組及卵巢切除組接受礦物油，共 6 週。分別於術後 2、4、及 6 週犧牲動物每組三隻，並於犧牲前一天及前七天分別注射綠及紅骨螢光標記。經擷取上、下顎骨標本：左、右之上顎骨標本分別以脫鈣及不脫鈣組織病理方法觀察骨生成及骨再吸收變化，而下顎骨標本則備製以作爲掃描電子顯鏡觀察。

結果：掃描電子顯鏡下觀察發現，控制組之下額骨體區呈正常皮質及骨小樑之結構，然而卵巢切除組及環孢靈組動物之骨小樑則較細、少。下額齒堉區之臼齒間與臼齒內其骨型態稍有不同：臼齒間之齒槽骨較密致並有小骨髓腔分散其中，而臼齒內之齒槽骨仍呈皮質包圍骨小樑之結構。在

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卵巢切除組及環孢靈組，其骨小樑仍較少且細，尤其是分布在臼齒間齒槽骨更爲明顯。上顎骨僅以四星期之標本爲限，卵巢切除組及環孢靈組動物齒槽骨骨體積雖下降但骨表面積卻增加。

若以螢光標記線間之距離分析骨沉積，卵巢切除組及環孢靈組動物之骨沉積較控制組者少，但三組間之骨吸收現象則較不明確。

建議：本實驗發現卵巢切除及環孢靈動物之下額骨骨小樑較細且少，且上顎無牙堉齒槽骨之骨沉積也較控制組低。因此我們推測卵巢切除及環孢靈藥物均可能誘導口腔齒槽堉骨質之缺乏或疏鬆，他們對骨生成之抑制可能與該病變有關。

關鍵詞：環孢靈（Cyclosporine A）、齒槽骨（Dental alveolus）　、骨質疏鬆（Osteoporosis）、卵巢切除術（Ovariectomy）

I、INTRODUCTION

Osteoporosis is a major clinical problem in older women and men. Almost any bone can fracture as a result of the increased bone fragility of osteoporosis. These fractures are associated with higher health care costs, physical disability, impaired quality of life （Srivastava and Deal, 2002）. Osteoporosis and osteoarthritis are two major health problems affecting more than 60% of post-menopausal women in the United States （Avci and Bachmann, 2004）. Hip fractures have high mortality and morbidity rate for the people as a complication of osteoporosis and are generally seen in old age （Calis *et al.*, 2004）. Osteoporosis in general affects one in four women over the age of 65 in the United States and is a major cause of hip fractures that place women in nursing homes （Ailinger and Emerson, 1998）. The incidence of pre-menopausal fractures within the last 10 years was 7.65 per 1000 person/years and that of post-menopausal fractures was 17.40 per 1000 person/years （Tuppurainen *et al.*, 1993）. Castration in pre-menopause had significantly decreased bone density, but no influence in mean levels of parameters of lipid metabolism, after the operation of overiectomy （Jenicek *et al.*, 2001）.The osteoporosis, therefore, has been considered as a serious global health problem for the future （Daniele *et al.*, 2004）.

Recently, it has been found that the osteoporosis and the related fractures are a major complication after organ transplantation （Leidig-Bruckner *et al.*, 2001）. After renal transplantation, osteoporosis is also a major source of morbidity （Ugur *et al.*, 2001）. The immunosuppressive drug cyclosporin A（CsA）has been thought to be involved in the pathogenesis of post-transplantation osteoporosis（Erben *et al.*, 2003）. CsA-treated groups demonstrated histological changes in some aspects resembling rickets/osteomalacia. （Abdelhadi *et al.*, 2002）. CsA induces high

turnover osteopenia in the rat and there is evidence for this in humans （Epstein *et al.*, 2001）. The ovariectomy in rats decreased significantly the content of calcium and magnesium of bones, while these minerals were increased after the estrogen therapy （Hempel *et al.*, 1978）. In human observations, the fractures due to the induced osteoporosis are occurring mostly during the first 6 months after the graft, with an incidence ranging from 18% to 50% for vertebral fractures （Krieg *et al.*, 2001）. Radius also seems to be one of the major parts of the skeleton affected by factors introduced after renal transplantation （Ugur *et al.*, 2001）. In our laboratory, the CsA-induced osteopenia can also be observed around the dental alveoli of the mandible of rats （Shen *et al.*, 2001）. However, the exact role of CsA in the development of osteoporosis and pathologic fractures frequently reported in patients following organ transplantation is still not known （Abdelhadi *et al.*, 2002）.

Women need information about the process of osteoporosis, the assessment of the individual fracture risk and the range of available options for the managements of the development of bone fragility （Unsworth, 2003）. However, the majority of women had inadequate knowledge of osteoporosis risk factors and preventive behavior （Ailinger and Emerson, 1998）. Nurses can play a major role in providing information, counseling, and developing decision aids（Theroux and Taylor, 2003）. Nurses also need to plan educational programs in all settings to teach older clients about the risk factors, prevention, diagnosis, and treatment of osteoporosis（Curry *et al.*, 2002）. The promotion of health of aging is a main concern for healthcare providers, including nurses. In concerning the oral health, the partial or even full edentulous due to loss their teeth are also commonly seen in aged women. Therefore, the purpose of the present study was to compare the dental alveolar bony alterations after the ovariectomy or cyclosporine administration in rats.

II、MATERIAL AND METHODS

Experimental Design:

Twenty-seven female Sprague-Dawley rats, five-week-old and weight 120-150gm, were purchased from the National Laboratory Animal Breeding and Research Center of the Taiwan National Science Council. The animals were caged in clear plastic cages and were kept in an environmentally controlled room maintained at 23^0C with relative humidity 55% and light-dark cycle of 12h/12h in Animal Center of National Defense Medical Center, Taipei, Taiwan. Before the experiments, all animals were received the right and left maxillary molars extraction under general anesthesia using an intraperitoneal injection of ketamine, 0.1ml/100g body weight （Hsieh *et al.*, 1994）. The hemostases of the sockets were achieved for 5 minutes with cotton pellet. The animals were then randomly designed into three groups: the control, ovariectomy and CSA groups. The nine rats of the ovariectomy group had the surgical ovariectomy operation done, while the other rats received only sham operation. After a three-week healing period, the rats in CsA group daily received CsA （Sandimmun, Sandoz, Basel, Switzerland）, 15 mg/kg body weight, by gastric feeding for 6 weeks, whereas the rats in the control and ovariectomy group received only mineral oil - the solvent. Three animals per group were sacrificed by carbon dioxide inhalation at the end of week 2, 4 and 6. The maxillary edentulous ridges on the right-hand side were dissected and fixed in 70% ethanol immediately for undecalcified histological preparation. After embedded in methyl methacrylate, the specimens were serially sectioned in frontal plane at 8 μm width, and stained with toluidine blue. The histological examination utilized the tissue sections from the mid-aspect of the edentulous ridge around the secondary molar region according to

the dental and the nasal anatomy landmarks （Hsieh *et al.*, 1994）. The maxillary ridges on the left-hand side were also dissected and fixed in 10% neutral formalin. After EDTA decalcification, the specimens were then processed as routine histological preparation. For the bone resorption, the tartrate-resistant acid phosphatase （TRAP） staining was performed and the positively staining cells can be counted. Both right and left mandible ridges, with all molars, were fixed in 10% neutral formalin and stored for further morphological examination by scanning electron microscopy （SEM）. For each right and left mandible, there were two dental alveolar bony ridges （the inter- and intra-molar ridges） sectioned according to the relationship with the molars for SEM examination. In each selected ridge, there were two zones, the zone within the ranges of the molar roots （the zone of tooth ridge） and the other zone apical to apical of root （the zone of mandibular body）, were further carefully observed.

Evaluation of Bone Formation and Resorption:

For all animals, the bony markers, calcium green and alizarin red were intra-peritoneal injected at the seven days and one day before the sacrificing, respectively. By fluorescent microscopy, the red or green fluorescent lines were easily recognized on the undecalcified bony tissue sections and the bone formation, recognized by the width between two fluorescent lines, could be analyzed.

III、RESULTS

In the mandible body zone of control animals, the bony pattern consisted of the central bony marrow, filled with trabecular bone, and surrounding condensed cortex bone was observed with SEM （Figure 1-2）. In those of OVX and CsA groups, a loss and fine trabecular bone bony pattern were observed, although a clear condensed cortex bone was still existed, regardless the inter- and intra-molar ridges

（Figure 1-2）. In addition, the trabecular bony loss at the week-6 showed much worse than that at the week-2.

In the tooth ridge zone of control animals, a dense bony pattern with scattered small bone marrow spaces was found in the inter-molar ridge. However, the pattern of a surrounding cortex with the central trabecular bone was noted in the tooth ridge zone of intra-molar ridge. Further trabecular bone loss was observed in both OVX and CsA groups where compared to those of control group, especially in the inter-molar ridge （Figure1-2）. The bony loss showed worse following the longer observation intervals.

In the maxillary edentulous ridge, only the specimens obtained at week-4 were complete the examination of bone formation and resorption. In general, a normal intra-membranous bony pattern was noted which was quit different with that in the mandible （Figure 3A and D）. However, a decreased alveolar bone volume, but an increased bone-specific surface, was found in the dental alveolus from the OVX （Figure 3B and E） or CsA （Figure 3C and F） group than that of control group. By fluorescent microscopy, the bony markers of alizarin red and calcium green fluorescent lines could be easily recognized on the bony tissue sections for all animals （Figure 4A-C）. Bony depositions （the width between two fluorescent lines） showed less, but the depositional surfaces showed greater, in OVX or CsA group if compared with that in control group. The TRAP stain, representing the bony resorption, showed similar among the maxillary alveolus from three examined groups （Figure 4D-F）.

IV、DISCUSSION

In the present study, the comparisons of the dental alveolar bony alterations after the ovariectomy or cyclosporine-A administration in rats were examined for 6

weeks. By SEM, an osteopenia/osteoporosis in mandibular dental alveolus was observed in animals received OVX or CsA. In maxillary edentulous ridges, a reduced bone formation was observed in rats from OVX and CsA rats when compared with that from the control. The bone resorption, however, showed uncertain at least at the observation point at week-4. Therefore, an inhibitory effect of OVX or CsA on the edentulous alveolar bone formation was suggested. Studies have been shown that CsA inhibits osteoblast proliferation, cell number, mitogenesis, alkaline phosphates levels, and cell attachment in vitro （McCauley *et al.*, 1992）. And a significant decrease in serum alkaline phosphatase level was observed in rats treated with high CsA doses（50-100 mg/kg）（Bennett *et al.*, 1991; Thomson *et al.*, 1981a; Thomson *et al.*, 1981b）. However, observation of decreased bone resorption following CsA administration has also been reported（Orcel *et al.*, 1989）. Nevertheless, the metabolic heterogeneity among different bone （long bone, scapular and vertebrae） has been pointed out, and such evidence further aids in elucidating the controversy （Klein *et al.*, 1990）.

Recently, the trabecular changes in partially edentulous bone have also been evaluated in ovariectomized rabbits. The results of sparser trabecular structure, more trabecular osteoid, and increased trabecular bone turnover demonstrate mineralized bone loss in partially edentulous trabeculae of ovariectomized rabbit mandibles. Therefore, the authors suggested that the same loss in animals may occur in postmenopausal women （Cao *et al.*, 2004）. Based on a longitudinal assessment of changes in alveolar and skeletal bone mineral density （BMD） in ovariectomized animals, saliva has been suggested to be an adjunct screening method for assessment of skeletal bone density （Johnson *et al.*, 2002）. In the present study, we first demonstrated that the OVX- or CSA-induced osteopenia/osteoporosis could be observed at either maxillae or mandible, regardless the present of tooth. In support of

our study design that the evaluation on an edentulous ridge, a complete wound healing of tooth-extraction sockets at two weeks after operation in rats has been described in other studies. A dynamic alveolar bone remodeling in the adult rats was reported less than 2 weeks （Vignery and Baron, 1980）. Therefore, in the present study, the examinations of the effects of OVX and CsA on the maxillary edentulous ridges were conducted 3 weeks after the molar extraction, when the bony ridge had been fully healed.

In conclusion, the dental alveolar bony alterations after the ovariectomy or cyclosporine-A administration in rats were compared with control animals for 6 weeks. By SEM, an osteopenia/osteoporosis in mandibular dental alveolus and the bony body was observed in animals received OVX or CsA. In maxillary edentulous ridges, a reduced bone formation was further observed in rats from OVX and CsA groups; however, the bone resorption showed uncertain, at least at the observation point at week-4. On the limitation of study, we suggest that an osteopenea/osteoporosis in the oral skeleton can be induced by OVX or CsA and an inhibitory effect of OVX or CsA on the oral dental bone formation might be associated with the bony alterations.

V、ACKNOWLEDGMENT

This study was partially supported by the National Science Council, Taiwan, ROC （Grant No.NSC-92-2314-B-016-047）and the CY Foundation for Advancement of Education, Sciences and Medicine. The authors would express their great gratitude to Hsiao-Pei Tu, Yen-Teen Chen and Yu-Tang Chin for histotechnical preparation.

References

Abdelhadi M, Ericzon BG, Hultenby K, Sjoden G, Reinholt FP, Nordenstrom J （2002）. Structural skeletal impairment induced by immunosuppressive therapy in rats: cyclosporine A vs tacrolimus. *Transpl Int* 15（4）:180-7.

Ailinger RL, Emerson J （1998）. Women's knowledge of osteoporosis. *Appl Nurs Res* 11（3）:111-4.

Avci D, Bachmann GA （2004）. Osteoarthritis and osteoporosis in postmenopausal women: clinical similarities and differences. *Menopause* 11（6）:615-621.

Bennett VJ, Detmar J, Chang PL （1991）. Growth inhibition by cyclosporine A in vivo and in vitro. *Life Sci* 48（15）:1455-61.

Calis HT, Eryavuz M, Calis M （2004）. Comparison of femoral geometry among cases with and without hip fractures. *Yonsei Med J* 45（5）:901-7.

Cao T, Shirota T, Ohno K, Michi KI （2004）. Mineralized bone loss in partially edentulous trabeculae of ovariectomized rabbit mandibles. *J Periodontal Res* 39（1）:37-41.

Curry LC, Hogstel MO, Davis GC, Frable PJ （2002）. Population-based osteoporosis education for older women. *Public Health Nurs* 19（6）:460-9.

Daniele ND, Carbonelli MG, Candeloro N, Iacopino L, De Lorenzo A, Andreoli A （2004）. Effect of supplementation of calcium and Vitamin D on bone mineral density and bone mineral content in peri- and post-menopause women; A double-blind, randomized, controlled trial. *Pharmacol Res* 50（6）:637-41.

Epstein S, Dissanayake IR, Goodman GR, Bowman AR, Zhou H, Ma Y, Jee WS （2001）. Effect of the interaction of parathyroid hormone and cyclosporine A

on bone mineral metabolism in the rat. *Calcif Tissue Int* 68（4）:240-7.

Erben RG, Brunner KS, Breig B, Eberle J, Goldberg M, Hofbauer LC （2003）. Skeletal effects of cyclosporin A are gender related in rats. *Endocrinology* 144（1）:40-9.

Hempel E, Gunther P, Neumann G, Muller B （1978）. Experimental studies on the castration osteoporosis in the rat. II. Behavior of bone minerals. *Zentralbl Gynakol* 100（21）:1391-8.

Hsieh YD, Devlin H, Roberts C （1994）. Early alveolar ridge osteogenesis following tooth extraction in the rat. *Arch Oral Biol* 39（5）:425-8.

Jenicek J, Bendova M, Dohnalova A （2001）. Changes in lipid profiles in women after surgical castration during the fertile age. *Ceska Gynekol* 66（4）:236-9.

Johnson RB, Gilbert JA, Cooper RC, Parsell DE, Stewart BA, Dai X, Nick TG, Streckfus CF, Butler RA, Boring JG （2002）. Effect of estrogen deficiency on skeletal and alveolar bone density in sheep. *J Periodontol* 73（4）:383-91.

Klein L, Li QX, Donovan CA, Powell AE （1990）. Variation of resorption rates in vivo of various bones in immature rats. *Bone Miner* 8（2）:169-75.

Krieg MA, Seydoux C, Sandini L, Goy JJ, Berguer DG, Thiebaud D, Burckhardt P （2001）. Intravenous pamidronate as treatment for osteoporosis after heart transplantation: a prospective study. *Osteoporos Int* 12（2）:112-6.

Leidig-Bruckner G, Hosch S, Dodidou P, Ritschel D, Conradt C, Klose C, Otto G, Lange R, Theilmann L, Zimmerman R, Pritsch M, Ziegler R （2001）. Frequency and predictors of osteoporotic fractures after cardiac or liver transplantation: a follow-up study. *Lancet* 357（9253）:342-7.

McCauley LK, Rosol TJ, Capen CC （1992）. Effects of cyclosporin A on rat osteoblasts （ROS 17/2.8 cells） in vitro. *Calcif Tissue Int* 51（4）:291-7.

Orcel P, Bielakoff J, Modrowski D, Miravet L, de Vernejoul MC （1989）. Cyclosporin A induces in vivo inhibition of resorption and stimulation of formation in rat bone. *J Bone Miner Res* 4（3）*:*387-91.

Shen EC, Fu E, Hsieh YD（2001）. Effects of cyclosporin A on dental alveolar bone: a histomorphometric study in rats. *J Periodontol* 72（5）*:*659-65.

Srivastava M, Deal C （2002）. Osteoporosis in elderly: prevention and treatment. *Clin Geriatr Med* 18（3）*:*529-55.

Theroux R, Taylor K （2003）. Women's decision making about the use of hormonal and nonhormonal remedies for the menopausal transition. *J Obstet Gynecol Neonatal Nurs* 32（6）*:*712-23.

Thomson AW, Whiting PH, Blair JT, Davidson RJ, Simpson JG （1981a）. Pathological changes developing in the rat during a 3-week course of high dosage cyclosporin A and their reversal following drug withdrawal. *Transplantation* 32（4）*:*271-7.

Thomson AW, Whiting PH, Cameron ID, Lessels SE, Simpson JG （1981b）. A toxicological study in rats receiving immunotherapeutic doses of cyclosporin A. *Transplantation* 31（2）*:*121-4.

Tuppurainen M, Honkanen R, Kroger H, Saarikoski S, Alhava E （1993）. Osteoporosis risk factors, gynaecological history and fractures in perimenopausal women--the results of the baseline postal enquiry of the Kuopio Osteoporosis Risk Factor and Prevention Study. *Maturitas* 17（2）*:*89-100.

Ugur A, Guvener N, Isiklar I, Turan M, Erdal R, Haberal M （2001）. Osteoporosis after renal transplantation: single center experience. *Transplantation* 71（5）:645-9.

Unsworth J （2003）. Prevention of fractures in older people who fall. *Br J Community Nurs* 8（7）:308-13.

Vignery A, Baron R （1980）. Dynamic histomorphometry of alveolar bone remodeling in the adult rat. *Anat Rec* 196（2）:191-200.

Legends

Figure 1. Microphotographs present the bony patterns of mandible body and tooth ridge zones at the intra-molar ridge of a control, OVX and CSA rat by SEM.

Figure 2. Microphotographs present the bony patterns of mandible body and tooth ridge zones at the inter-molar ridge of a control, OVX and CSA rat by SEM.

Figure 3. Microphotographs present the bone morphology of maxillary edentulous ridge, obtained at the week-4, from a control, OVX and CSA rat （A-C: H & E stain, magnification X 5）. Decreased alveolar bone volume, but increased bone-specific surface, was found in the dental alveolus from the OVX（B and E）and CSA（C and F）groups than that of control group （D-E: H & E stain, F: toluidine blue stain, magnification X 50）.

Figure 4. Two fluorescent lines（alizarin red and calcium green）were observed on the bony section of the maxillary edentulous ridge form a control, OVX and CSA rat under fluorescent microscope （A-C: magnification X 50）. The microphotographs of D, E and F present the bone resorption of osteoblasts by TRAP staining （D: magnification X 100; E-F: magnification X 50）.

Figure 1.　Microphotographs present the bony patterns of mandible body and tooth ridge zones at the intra-molar ridge of a control, OVX and CSA rat by SEM

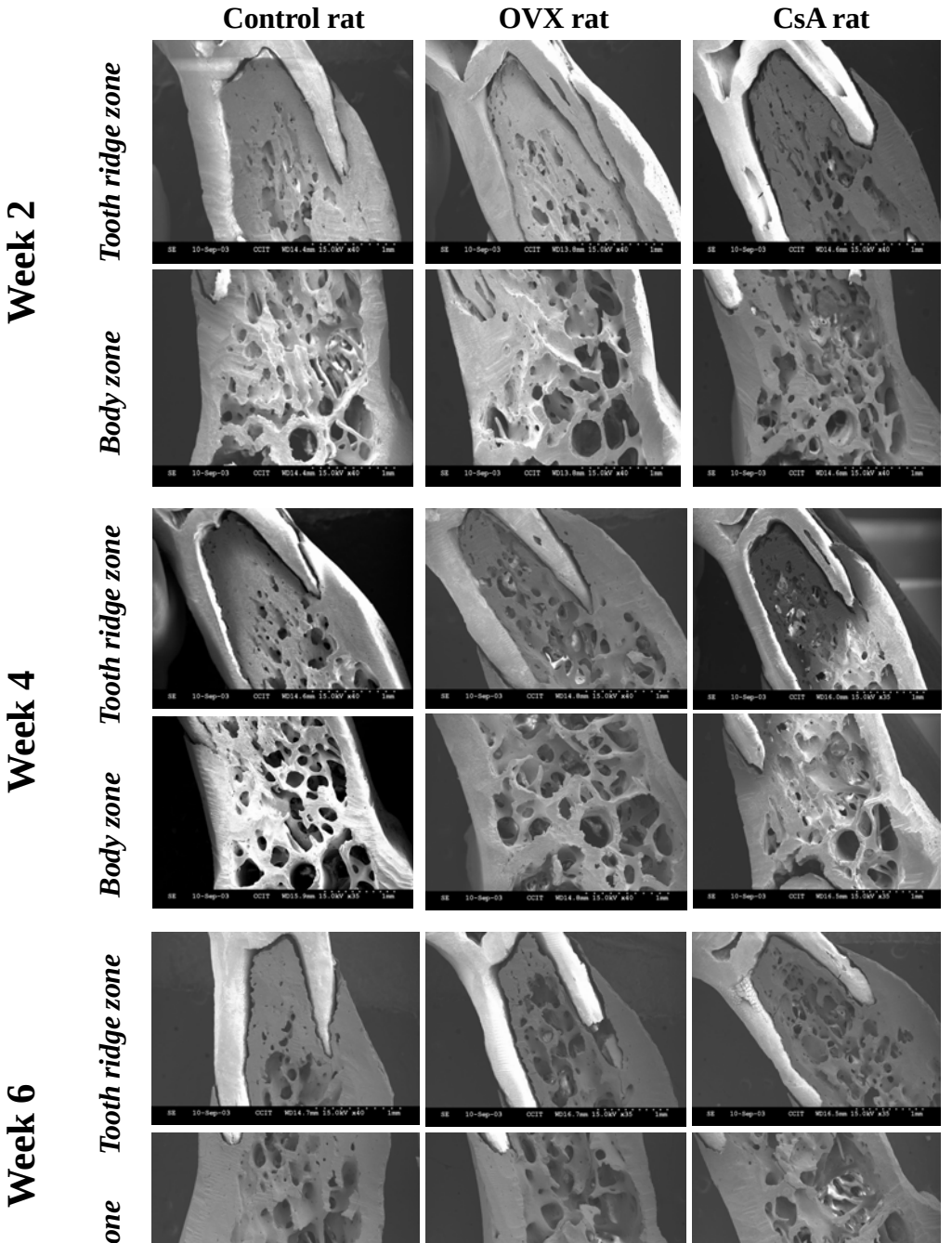

Figure 2. Microphotographs present the bony patterns of mandible body and tooth ridge zones at the inter-molar ridge of a control, OVX and CSA rat by SEM.

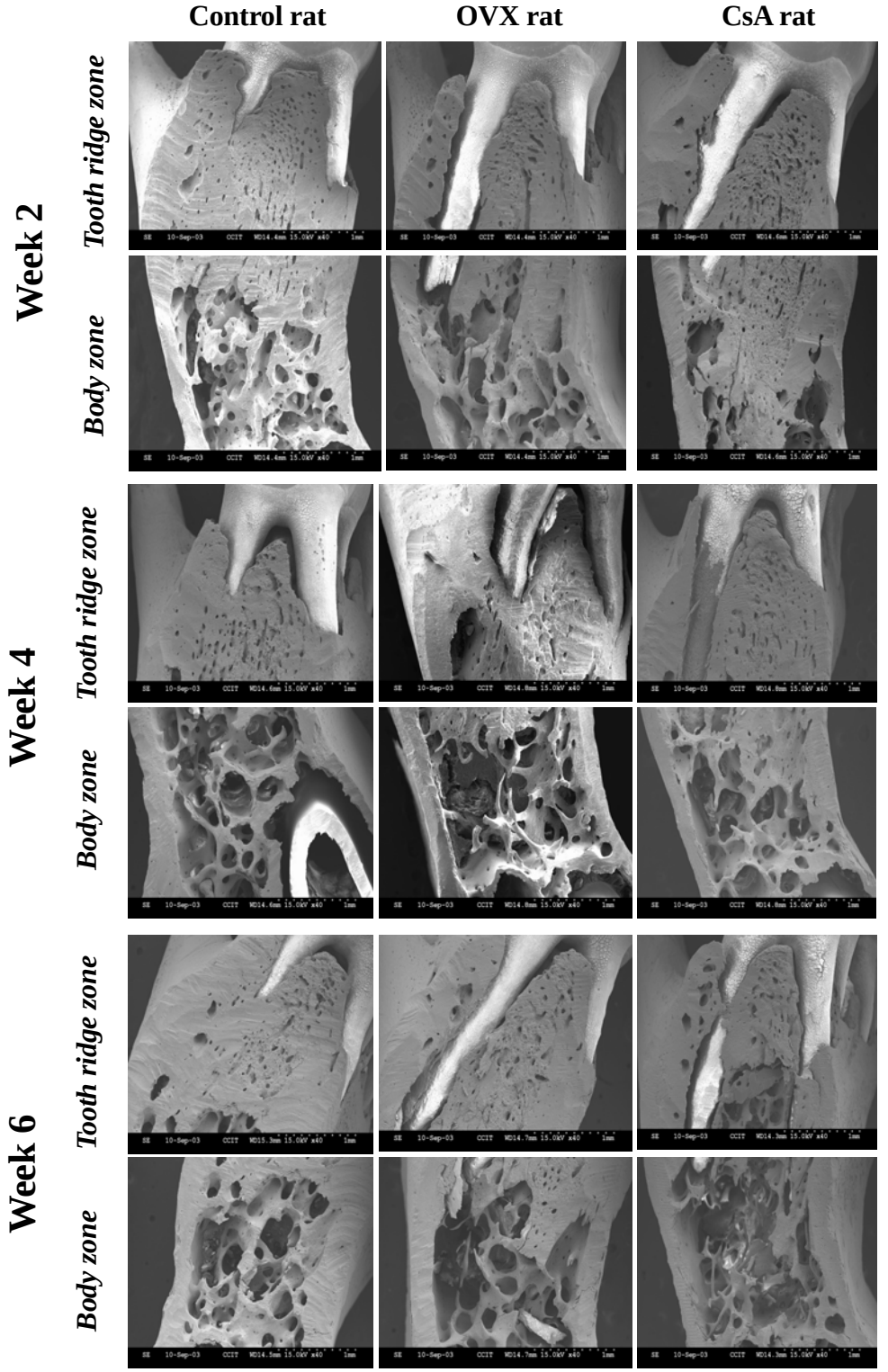

Figure 3. Microphotographs present the bone morphology of maxillary edentulous ridge, obtained at the week-4, from a control, OVX and CSA rat (A-C: H & E stain, magnification X 5). Decreased alveolar bone volume, but increased bone-specific surface, was found in the dental alveolus from the OVX (B and E) and CSA (C and F) groups than that of control group (H & E stain for D-F, magnification X 50).

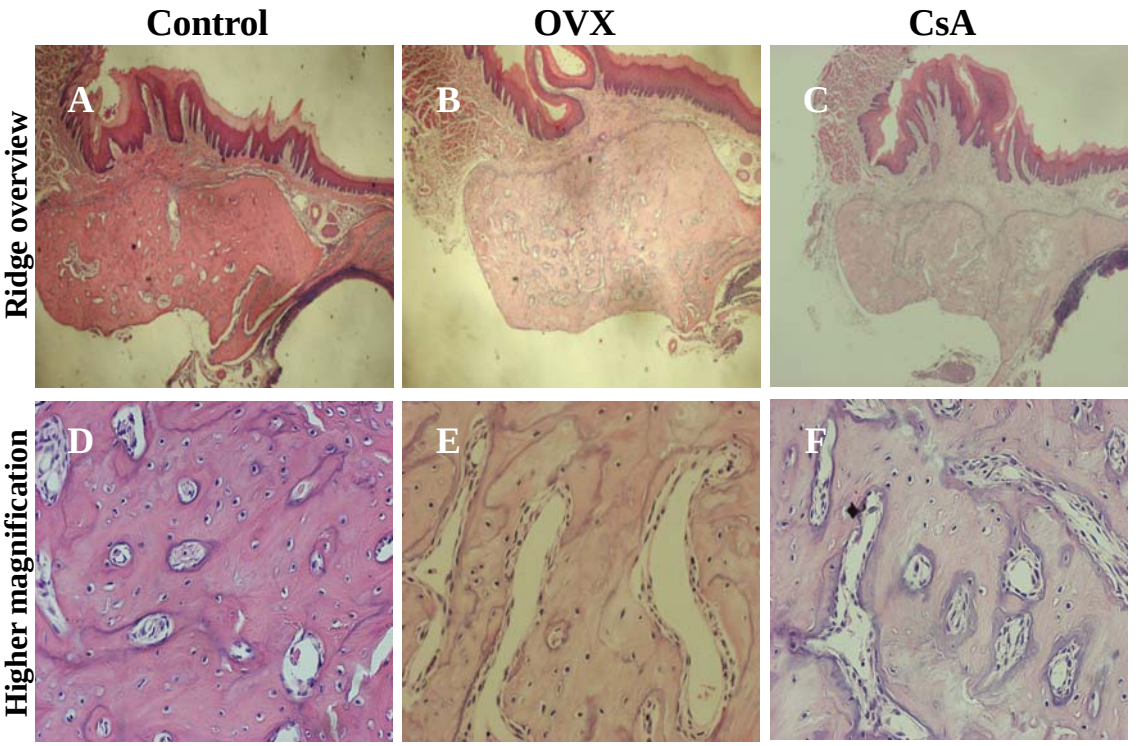

Figure 4. Two fluorescent lines (alizarin red and calcium green) were observed on the bony section of the maxillary edentulous ridge form a control, OVX and CSA rat under fluorescent microscope (A-C: magnification X 50). Microphotographs of D, E and F present the bone resorption of osteoblasts by TRAP staining (D: magnification X 100; E-F: magnification X 50).