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Pentoxifylline and tocopherol treatment improved the cortical bone quality and quantity on irradiated rodent model: A micro-CT and immunohistochemical study

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Truc Thi Hoang Nguyen의 석사학위논문을 인준함

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Abstract

Pentoxifylline and tocopherol treatment improved the cortical bone quality and quantity on irradiated rodent model: A micro-CT and immunohistochemical study

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Introduction

The use of radiotherapy (RT) in head and neck malignancies treatment includes primary therapy, RT as adjuvant to surgery and RT in combination with chemotherapy or as palliative treatment for late stage and unresectable tumors. With recent advances in radiation technique, the undesired effects of irradiation were minimized. However, patients still suffer from reactions of the mucosa, jaw bone or salivary glands, of which one specific adverse effect is osteoradionecrosis (ORN) of the mandible. Pentoxifylline and tocopherol together work as potent antifibrotic agents, which are believed to have the effect of reducing the chronic fibrosis and induce bone healing in ORN. This combination may provide an adjuvant to surgical treatment or an alternative noninvasive treatment for ORN. The study was designed to

assess the improvement of cortical bone quality and quantity on irradiated animal model treated with pentoxifylline and tocopherol using a novel micro-CT paradigm.

Materials and methods

Forty-eight 8-week-old male Sprague-Dawley rats (OrientBio Inc., Seongnam, Korea) with an average body weight of 369.6g were used in this study. Rats were randomly divided into irradiated group (n = 40) and non-irradiated control group (n = 8). In irradiated group, animals were divided into 4 subgroups according to treatment, including PTX + TP (pentoxifylline and tocopherol), PTX (pentoxifylline), TP (tocopherol), and NS (normal saline). Medicine was administrated until the end of the study. Three weeks after irradiation, mandibular posterior teeth were extracted. Animals were sacrificed 7 weeks after irradiation and 4 weeks after extraction. Cortical bone quality and quantity were analyzed using micro-CT and histological evaluation. Alveolar bone height (ABH) and cortical bone thickness (CBT) were compared between non-irradiation control group (C) and irradiated, normal saline administrated group (NS). ABH and CBT were also compared between irradiated groups with different medicine administrated. Cortical bone defect was evaluated on 3D reconstructed images.

Results

1. Micro-CT analyses

Both ABH and CBT results in group NS were significantly lower than those in non-irradiation group. The ABH and CBT of PTX + TP administrated group were significantly higher than other groups. The ABH and CBT of PTX and TP groups did not differed significantly. The CBT of PTX group was significantly higher than normal saline group ($p < 0.05$).

In all irradiated groups, alveolar bone loss in the lingual side (ΔHL) results were significantly higher than alveolar bone loss in the buccal side (ΔHB). In addition, on buccal side, ΔHB of PTX + TP group was significantly lower compare to PTX group and NS group. On the lingual side, ΔHL of NS group was significantly higher than ΔHL of PTX and PTX + TP group ($p < 0.05$).

In the irradiated group, cortical bone volume (Ct.BV) and total cortical bone surface (Ct.S) of PTX + TP group was significantly higher than which of PTX, TP and NS groups. Average cortical bone thickness (Ct.Th) of PTX + TP group was significantly higher than NS group. The total pore volume (PoV) of 4 groups did not differ significantly. However, total pore volume rate (PoV%) in PTX + TP group was significantly lower than which in PTX and NS groups, and did not significantly differ to TP group ($p < 0.05$).

2. Three-dimensional (3D) reconstructed images evaluation

In all four irradiated groups, observed defective state of lingual walls were more severe than defective state of buccal walls. Also, defective state of PTX + TP group was lower than PTX, TP and NS groups. The 3D reconstructed images were also used to evaluate the correlation between cortical bone defect and cancellous bone defect at the extracted sites. In non-irradiated group, bone healing occurred and the extracted socket was filled with new formed cancellous bone, surrounding by healthy cortical bone walls. In irradiated groups, the non-healing wound and bone necrosis were observed. In addition, there was an inadequacy in the residual volume of cortical bone and cancellous bone.

3. Histological and immunohistochemical analyses

We observed empty lacunae with loss of nuclei in irradiated groups, especially in NS group. Viable blood vessels within Haversian canals can be

observed in PTX + TP group, and a small amount in PTX group. In TP and NS group, the unviable blood vessels within Haversian canals were dominated.

CD31 (PECAM) staining was used for evaluation of microvessel density (MVD). MVD of PTX + TP group was significantly higher than which of PTX, PT and normal saline groups ($p < 0.05$). Osteocalcin (OC) staining was used for evaluation of osteocyte within cortical bone. Number of OC+ osteocyte in NS group was significantly lower than other three groups. Number of OC+ osteocytes in PTX + TP, PTX and TP groups did not differ significantly ($p < 0.05$). The expression of TNF-α and TGF-β1 was relatively lower in group taking PTX +TP. The other markers including IL-6, ALP did not show significant difference.

Conclusion

From the results our study, cortical bone has proven to play an important role in the evaluation and prognosis of ORN. The combination of PTX and TP has improving effect on the cortical bone quality and quantity, resulting in a regression of the disease and can be a reliable treatment protocol or used for early ORN prevention in particular susceptible patients.

주요어 : Osteoradionecrosis, pentoxifylline, tocopherol, micro-CT, cortical bone

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Content

I. Introduction

With recent advances in radiation techniques in the treatment of orofacial malignancy, the undesired effects of irradiation have been minimized. However, patients still suffer from irradiation reactions of the mucosa, jaw bone or salivary glands, of which one specific adverse effect is osteoradionecrosis (ORN) of the mandible [1-3]. The exact pathogenesis of ORN remains a matter of controversy. In 1983, Marx initiated the 3H theory stating that hypoxia, hypovascularity, and hypocellularity collectively lead to ORN [2, 4]. Twenty years later, in 2004, Delanian *et al*. introduced the radiation-induced fibroatrophic (RIF) theory [8]. According to this theory, the RIF process includes free radical formation, which leads to endothelial dysfunction, inflammation, microvascular thrombosis, fibrosis, and remodeling, and finally result in bone and tissue necrosis [4, 5]. The research group of Delanian then developed a treatment protocol which is a combination of antioxidant agents that works via the inhibition of free radical species. In a phase II trial, the combination of pentoxifylline (PTX) and tocopherol (TP) showed a positive effect on ORN treatment and a regression of the disease [6].

While PTX and TP are not new medicine, their benefits are still being investigated. Pentoxifylline is a methylxanthine derivative that has antioxidant properties (Appx, Fig. 1A). It inhibits the transcription of tumor necrosis factor-alpha (TNF-α) and reduces the cytokine cascade driving the process of ORN. In addition, PTX also improves the erythrocyte deformities, which result in enhanced blood flow [6-8]. There are several forms of tocopherol including alpha, beta, gamma, and delta forms [9]. Alpha-tocopherol (Appx, Fig. 1B), commonly known

as vitamin E, has antioxidant properties and it scavenges reactive oxygen species (ROS) that induce lipid peroxidation in cell membranes. Lipid peroxidation is involved in ORN pathogenesis, and therefore alpha-tocopherol protects cell membranes from the oxidative stress induced by radiation [4]. PTX and TP together work as potent antifibrotic agents, which are believed to have the effect of reducing the chronic progressive septic changes associated with ORN of the mandible [6, 10-12]. This combination may provide an adjuvant to surgical treatment or an alternative noninvasive treatment for ORN.

The effects of irradiation on the bone's composition have been studied in the few past decades since the disease was first reported. Several studies have pointed out that the blood deficiency and necrosis of the irradiated mandible are related to the lack of micro-vascular structure rather than to defects of large vessels [13, 14].

Despite the increasing incidence of ORN in patients treated with radiation therapy, little research is available to emphasize the importance of cortical bone remodeling. During screening and treatment of ORN patients, some noticeable differences in the destruction of cortical bone and cancellous bone were observed. The pattern of defection in which the cortical bone has the lower defective rate than the cancellous bone is different from other osteomyelitis diseases. In a BRONJ patient (Appx, Fig. 2), both cortical walls also have a similar destructive rate as the cancellous bone, which results in a large defect after saucerization surgery. On the other hand, in a patient with complex ORN defects (Appx, Fig. 3), the defects appeared on the right and left side of the mandible, with the different general level of destruction of both the cortical and cancellous bone. In another progressive mandibular ORN patient (Appx, Fig. 4), CT views showed that the destruction rate

of cortical bone was not the same as the cancellous bone. After saucerization surgery, a part of the cortical wall showed spontaneous bleeding and vital signs and therefore was preserved. From these observations, we have a hypothesis that there is a significant difference in the necrosis and destructive rate between irradiated mandibular cortical and cancellous bone, and that the cortical bone can play an important role in the clinical evaluation and prognosis of ORN.

Recently, many innovative and well-developed bone microarchitecture and morphometric parameters study methods have been introduced, such as peripheral Quantitative Computed Tomography (pQCT), Dual Energy X-ray Absorptiometry (DXA) and micro-Computed Tomography (micro-CT). Among those methods, micro-CT has been widely used to study the mandible, mainly in cancellous bone, and their remodeling after irradiation. However, like the protocols for studying cortical bone remodeling in rodent ORN mandibles, the literature is particularly scarce and the methodology for a comprehensive and reproducible quantitative analysis is not sufficiently informative or detailed [15].

The study was designed to assess the improvement of cortical bone quality and quantity on irradiated rat model administrated with pentoxifylline and tocopherol using a novel micro-CT paradigm. The goal of our study was to establish a new animal models of ORN by using the most current technology and updated knowledge regarding the disease process.

II. Materials and Methods

The timeline of the study started with animal preparation and grouping. One day after that, the radiation was administrated on the design irradiated groups. The medicine administration started one day after radiation. Three weeks after radiation, the first and second molars on the left mandible of each rat were extracted. The medicine was given uninterrupted to the designed groups. Four weeks after tooth extraction, the animals were sacrificed (Figs. 1,2).

1. Animal

Forty-eight 8-week-old male Sprague-Dawley rats (OrientBio Inc., Seongnam, Korea) with an average body weight of 369.6 g were used in this study. The study procedures received the animal research ethics approval from the Seoul National University Institutional Animal Care and Use Committee (SNU-180213-1-1). The rats were housed in the Laboratory Animal Center of the Korea Institute of Radiological and Medical Sciences, and were given ventilated cages, standard 12-hour light/dark cycles and had access to chow and water ad libitum. The rats were randomly divided into an irradiated group (40 rats) and a non-irradiated or control group (8 rats). In each group, the rats were divided into four small groups according to the dosed medicine. The irradiated group included PTX +TP (pentoxifylline and tocopherol), PTX (pentoxifylline), TP (tocopherol) and NS (normal saline). The non-irradiated group included 8 rats and was designated as group C (Table 1). After irradiation with high radiation doses (35 Gy), 4 animals in the irradiated group died and one animal was released from anesthesia during irradiation and was excluded from the study.

2. Radiation procedures

The radiation was performed under anesthesia with an intraperitoneal injection of ketamine (60 mg/kg, ketamine hydrochloride®; Yuhan Co., Seoul, Korea) and Xylazine (3 mg/kg xylazine hydrochloride®; Bayer Korea, Seoul, Korea). The external radiation procedure was performed with an X-RAD 320 Irradiator® (Precision X-ray Inc., North Branford, USA). The irradiator can provide a high output uniform beam with the 320kV X-ray tube. The single radiation dose was 35Gy with the dose rate was 2.5Gy/minute. The animals were positioned on their right side, the irradiated area was defined as a 1 x 2-cm rectangular area on the left mandibular body and the isocenter was located using laser light (Fig. 2A).

3. Medication, extraction and sacrifice procedures

The day after irradiation, daily oral medication to rats according to each defined experimental group was initiated. PTX (Trental® 400mg, Handok Co., Seoul, Korea) was administered daily with a dose of 50 mg/kg, TP (Yuhan Co., Seoul, Korea) was administered at 40 IU/kg daily using an oral gavage (Fig. 2B). All rats underwent atraumatic extraction of the first and the second left mandibular molars three weeks after irradiation (Fig. 2C). The surgical procedures were performed under general anesthesia, including dermal and oral disinfection with betadine, gingiva detachment and atraumatic extraction. No suture was performed. The rats were maintained on soft chow and water ad libitum.

The animals were sacrificed seven weeks after irradiation and four weeks after extraction with CO_2 inhalation. The mandible was isolated, all soft tissue was removed then the mandibular bone was fixed in formalin (Fig. 2D).

4. Micro-CT scanning and data reconstruction procedures

Micro-CT was performed using a high-resolution micro-CT scanner Skyscan 1172® (Bruker Co., Kontich, Belgium). For scanning preparation, the mandible was placed in a polyethylene tube with a soft clay layer to keep the specimen in a fixed position. The tube was then filled with normal saline. The scan parameters were 70 kV, 141 µA, with a 0.5-mm Al filter, undergoing a 360° rotation with 0.4° steps. The cubic voxel size in the output three-dimensional images is 16.94 µm.

The data sets were reconstructed with NRecon software® (SkyScan Co., Aartselaar, Belgium) with the adjustment of reconstruction parameters, including beam-hardening factor correction, ring artifact reduction, and smoothing. The quantitative information of the bone structure was assessed using a data viewer and the CtAn Image Processing Language® (Bruker Co., Kontich, Belgium) (Fig. 3).

5. Micro-CT analyses

The VOI included the entire defected volume in the first and the second molar region post-extraction, with a fixed data set of 360 slices. The ROI was defined as the entirety of the cortical bone the mandible and was managed semi-manually (Fig. 4). After manual delineation matching the cortical bone area, the ROIs were then interpolated for the next slices and were continuously checked and re-delineated to be sure that no cortical bone was cropped out.

The cortical bone-related parameters, including the total cortical bone volume (Ct.BV, mm^3), average cortical bone thickness (Ct.Th, mm), cortical bone surface (Ct.S, mm^2), total volume of pore space (Po.V, mm^3) and the rate of porosity (%) were measured.

Alveolar bone height (ABH) and cortical bone thickness (CBT) measuring method were referred from radiographic and histometric study of Guglielmotti *et*

al.[16]. In the Dataview window, one slice, which was located in the middle of the first molar teeth, was chosen. (a) is the line drawn tangential to the upper cortical border of the mandibular canal at C and is perpendicular to the external surface of the buccal plate (b). Point A is at the top of the vestibular crest, point A' is the point of intersection of the line drawn from A to (a) that is perpendicular to line (a). Point B is at the top of the lingual crest, B' is the point of intersection of line drawn from A that is perpendicular to the line (a). AA' (h1), BB' (h2) is the alveolar bone height on vestibular and lingual side, respectively. The ABH and CBT on both the buccal and lingual side of the extracted and non-extracted jaw were assessed and measured using CTAn® and ImageJ® (U.S. National Institute of Health, Bethesda, MD, USA). (Fig. 5).

Three-dimensional (3D) images of mandibular bony defects were reconstructed with CTVol software® (SkyscanVR, Kontich, Belgium). The intracortical porosity and canal system were visualized using CTAn and CTVol.

6. Histological and immunohistochemical evaluation

For histological staining, decalcified paraffin sections were cleaned with xylene for 10 minutes and 4-μm thick slices were prepared and then stained with both H&E and MT.

For immunostaining, paraffin-embedded tissues were sectioned at 4-μm intervals and collected in serial sections on slide glass. An automated BOND-MAX system® (Leica Microsystems, Mannheim, Germany) was used. The used IHC markers include TNF-α, TGF-β1, IL-6, ALP, OC, and CD31. For evaluation of microvessel density (MVD), slides were scanned and the "hot spots" were identified at ×40 magnification and the number of CD31+ microvessels was

counted at ×200 magnification per measurement field [17]. Counting was performed in two different fields of cortical bone. Osteocalcin (OC) staining was also screened for osteocytes at ×400 magnification. For other antibodies, staining was scored as follows: negative, weak (1-10% of cells positive), moderate (11-50% of cells positive), and strong (>50% of cells positive).

7. Statistical analysis

The means and standard deviations (SDs) of alveolar bone loss, cortical bone thickness and other cortical bone parameters were calculated. The Shapiro–Wilk test was used to test the normality of variables. The differences between follow-up periods were tested by ANOVA. All analyses were carried out using SPSS (SPSS 25.0®; SPSS Software Company, Chicago, IL, USA). P values < 0.05 were considered statistically significant.

III. Results

We observed an area of depilation in the irradiated mandible, and the other alterations on teeth were also recorded including stopped growth of mandibular incisors and dental fractures which were observed in three animals. At the extraction zone in the irradiated groups, the non-healing mucosal wounds were observed. (Fig. 6).

1. Alveolar bone height (ABH) and cortical bone thickness (CBT) evaluation

Alveolar bone heights and cortical bone thicknesses were compared between the non-irradiation group (C) and irradiated, NS administrated group (Table 2). Both ABH and CBT results in group NS were significantly lower than the ABH and CBT in the non-irradiation group. In irradiated groups, The ABH and CBT of the PTX + TP administered group were significantly higher than in the other groups. The ABH and CBT of the PTX and TP groups did not significantly differ. The CBT of the PTX group was significantly higher than the NS group ($p < 0.05$) (Tables 3,4).

The alveolar bone loss was defined as the difference of ABH between the irradiated jaw and opposite jaw and designated as ΔHB and ΔHL for the buccal side and lingual side, respectively (Table 3). In all irradiated groups, ΔHL results were significantly higher than ΔHB. In addition, on the buccal side, the ΔHB of PTX + TP group was significantly lower compared to the PTX group and NS group. On the lingual side, the ΔHL of NS the administrated group was significantly higher than the ΔHL of the PTX and PTX + TP administered group ($p < 0.05$).

2. Cortical bone parameters analyses

In the irradiated group, the cortical bone volume (Ct.BV) and total cortical bone surface (Ct.S) of the PTX + TP group were significantly larger than those of the PTX, TP and NS groups (Fig. 7,8). The average cortical bone thickness (Ct.Th) of the PTX + TP group was significantly higher than that of the NS group (Fig. 9). The total pore volume (PoV) of the four groups did not differ significantly (Fig. 10). However, the total pore volume rate (PoV%) in the PTX + TP group was significantly lower than that of the PTX and NS groups and did not significantly differ from the TP group ($p < 0.05$) (Fig. 11).

3. Three-dimensional (3D) reconstructed images evaluation

Cortical bone defects were evaluated using 3D reconstructed images (Fig. 12). In all four irradiated groups, lingual wall defection was relatively higher than the buccal wall defection. Also, the defective state in the PTX + TP group was lower than that those of the PTX, TP and normal saline groups (Figs. 13,14). The 3D reconstructed images were also used to evaluate the correlation between the cortical bone defects and cancellous bone defects at the extracted sites. In the irradiated groups, non-healing wounds and bone necrosis were observed. In addition, in the irradiated groups, there was an inadequacy in the residual volume of cortical bone and cancellous bone. The 3D reconstruction of intracortical bone porosity and vessel canals showed a relative higher bone vascularity in PTX+TP groups (Fig. 12).

4. Histological and immunohistochemical analysis

We observed empty lacunae with loss of nuclei in the irradiated groups, especially in the NS group. Viable blood vessels within Haversian canals could be observed in the PTX + TP group, and to a smaller extent in the PTX group. In the TP and NS groups, the presence of non-viable blood vessels within Haversian canals dominated (Fig. 15). CD31 (PECAM) staining was used for the evaluation of microvessel density (MVD) (Table 6). The MVD of the PTX + TP group was significantly higher than that of the PTX, PT and NS groups ($p < 0.05$) (Fig. 16). Osteocalcin (OC) staining was used for the evaluation of osteocytes within cortical bone (Fig. 17). The number of OC+ osteocytes in the NS group was significantly lower than in the other three groups. The number of OC + osteocytes in the PTX + TP, PTX, and TP groups did not differ significantly ($p < 0.05$). The expression of TNF-α and TGF-β1 was relatively lower in the group taking PTX +TP. The other markers, including IL-6 and ALP, did not show significant differences (Table 6).

(histological result discussion)

IV. Discussion

Osteoradionecrosis is a severe radiation-induced complication in head and neck region. ORN manifestation is described as an exposed irradiated bone that fails to heal within 2 months with the presence of devitalized bone, with or without orocutaneous fistulas. ORN lesions can appear from months to a year post-irradiation and have a diversity of presentations, ranging from intraoral bone exposure to extraoral draining fistulas and even to pathological mandibular fractures. Among the several suggested classifications, the classification of ORN by Notani *et al*. is simple and widely used [6].

On 1983, after the introduction of 3H theory (hypoxic – hypocellular – hypovascular) of Marx (Appx, Fig. 5), Mainous *et al*. suggested the use of hyperbaric oxygen (HBO) in ORN management [2, 4, 18]. However, the affection of HBO on ORN tissues is still under controversy. In 2005, Benett *et al*. reviewed the HBO therapy for late radiation injury of tissue (LRIT) on Cochrane Collaboration. The authors reported that there was a significant improvement in healing of tooth sockets following dental extraction in irradiated field, however the evidences were limited by small numbers of participants, poor reporting of methods and results in lacking of defined exact degree of defect improvement with HBO. According to a recent review of Bennett et al. on Cochrane (2016), there was moderate quality evidence of a significant improved chance of wound breakdown without HBO therapy following operative treatment for ORN [19]. With the development of new protocols, HBO therapy now can be recommended as an adjuvant therapy and the economic aspect should be considered.

In 2004, Delanian *et al*. introduced the radiation-induced fibroatrophic process

(RIF) (Appx, Fig. 6) [8]. The authors then developed an etiological treatment therapy which is a combination of antioxidant agents that works on the inhibition of free radical species. In a phase II trial performed by the same authors group, the combination of pentoxifylline and tocopherol showed the positive effect on ORN treatment and a regression of disease [6]. In this clinical study, all patients had a history of failed ORN treatment with antibiotics, HBO, and/or sequestrectomy. The study included two different treatment protocol: PTX + TP (10 patients), PTX + TP and clodronate (8 patients). In the severe cases, antibiotics and steroids were also administrated. Among 18 patients included in the study, 16 patients recovered completely, and of these, 14 patients had the rapid complete recovery in a mean 5.2 ± 2.6 months. McLeod *et al.* in 2012 reported their experience evaluating ORN treatment protocol with pentoxifylline 400 mg twice daily and vitamin E 1000 IU daily using the SOMA score and Epstein stage classification [20]. In an average treating period of 46 months (range 4– 46 months of treatment) the SOMA score improved in eight patients, and the Epstein stage improved in five of 12, worsened in two of 12, and remained the same in five of 12 patients. In a systemic review and meta-analysis in 2018, Kolokythas *et al.* measured and reported the full recovery or significant improvement in ORN patients treated with pentoxifylline and tocopherol without surgical intervention [21]. The study's result demonstrated successful outcomes with the use of antioxidant therapeutic in ORN treatment, especially pentoxifylline and tocopherol. The recovery or significant improvement rate in patient treated with pentoxifylline and tocopherol was up to 60%. The regression of ORN process and therefore did not require surgical treatment was experienced in 28% of the patients. Seven percent of ORN cases treated with PTX

and TP required further surgical treatment. PTX is primarily an agent for the treatment of peripheral vascular disease that works by enhancing red blood cell deformability and increasing microcirculation [22]. In recent studies, the action of PTX in inhibiting SMADs, a group of proteins that transduce extracellular signals from the transforming growth factor-beta 1 (TGF-β1) to the nucleus, led to the inhibition of SMAD-dependent transcription of either the fibrotic proteins or the antifibrotic action of PTX [7]. PTX is also the inhibitor of the tumor necrosis factor - alpha (TNF-α), interleukin - 1(IL-1) and the fibroblast growth factor (FGF) [23]. TP acts as an antioxidant and scavenges free radical agents. It is also reported to have role in the reduction of fibrosis and cell apoptosis by its action on TGF-β1, intercellular adhesion molecular 1 (ICAM-1), and proliferator activated receptor-gamma (PPAR-γ) [23]. Many authors have been studied the mechanism of the PTX-TP combination in the prevention and treatment of ORN, however, the precise complex interaction is still under controversy. Recently, the PTX-TP combination has been considered as a useful protocol for irradiated patients. Delanian *et al.* reported a reduction of ORN when PTX (800mg/day) and TP (1000 mg/day) was given for 6 months [24, 25].

From recent studies, the cortical bone is considered an important target for bone quality assessment and pharmacological treatment [26]. Bagi *et al.* [27] suggested that micro-CT imaging of the jawbone in a rodent can be a reliable methodology for the assessment of the cortical bone morphology, micro-architecture and mineral density. In addition, micro-CT provides an instrument to evaluate the progress of bone defect healing and the process of remodeling bone growth into the defect in 2D and 3D planes. Another advantage of the micro-CT

technique over histology is that the microradiographs can be easily obtained, in a relatively short period of time and at low cost [28].

In this animal study, to create an ORN model, we used the radiation rate of 35Gy, 2.5Gy/min, on a 2 x 1-cm^2-sized surface of the mandible. According to Hopewell, the reduce in bone blood-flow is dose-related and after singles doses of > 20Gy, changes in bone mineral content was found [29]. Niehoff et al. also demonstrated a single dose of 20Gy can reduce the bone regeneration in rat mandible [30]. In our study, the single dose of 35Gy was applied and all the irradiated groups showed the delayed intraoral wound healing and skin alopecia at the irradiated site. In addition, the finding of necrosis bone in micro-CT images of all irradiated groups also strengthen the establishment of radiation-induced osteonecrosis in our animal model.

In this study, micro-CT analysis tools provided a repeatable measurement of the cortical bone parameter in the animal experiment and also detected morphological changes and ORN's manifestation. The alveolar bone heights of each jaw on both the buccal and lingual side were measured. In the non-irradiated group, the difference between the alveolar bone height (ABH) of the extracted jaw and the ABH of opposite jaw on the buccal side and lingual side did not significantly differ between the four groups ($p < 0.05$). In all irradiated groups, the ΔHL results were significantly higher than ΔHB. This difference between the interfered jaw and non-interfered jaw can be considered as the result of cortical bone loss due to extraction alone or due to the extraction on the irradiated jaw. These generally results suggested the effect of radiation on the cortical bone, however, the result that the cortical bone loss on the lingual bone wall is higher than in the buccal bone wall is

still unclear. In our point of view, this difference may be because of the anatomic and original bone thickness of the jaw bone. The lingual one wall is usually thinner than the buccal bone wall. Further studies need to be performed to confirm this result.

Other cortical bone parameters also offer support for the treatment using PTX and TP combination. The Ct.BV of the PTX + TP group was significantly higher than those of the PTX, TP and NS groups. The Ct.Th of the PTX + TP group was significantly higher than of the NS group. The Ct.S of the PTX + TP therapy was significantly higher than of the PTX, TP and NS groups. The PoV of the 4 four groups did not differ significantly. However, the PoV% in the PTX+TP group was significantly lower than PoV% in other groups. Interestingly, in 3D reconstructed images, porosity and vessels canal amount in PTX+TP groups was relative higher than that of other groups. These results can be explained by the volume of open porosity, which was excluded in the intravascular 3D analysis. The low level of open porosity in PTX+TP groups support for the evidence of bone healing effect of this combination.

CD31 (PECAM) is a single chain type-1 transmembrane protein that plays a role in the adhesive interactions between adjacent endothelial cells, as well as between leukocytes and endothelial cells [31]. It has recently been recognized for its angiogenic role [32, 33] and therefore it was chosen for the microvessel density (MVD) analyses. OC and ALP is known to be a bone tissue-specific protein [34] and was used to evaluate cortical bone remodeling. Other markers, including TNF-α, TGF-β1, and IL-6, were used to evaluate the inflammation within the cortical bone. The MVD of the PTX + TP group was significantly higher than those of the

PTX, PT, and NS groups ($p < 0.05$). The number of OC+ osteocytes in the NS group was significantly lower than the other three groups. The number of OC+ osteocytes in the PTX + TP, PTX, and TP groups did not differ significantly ($p < 0.05$). The expression of TNF-α and TGF-β1 was relatively lower in the group taking PTX + TP. The other markers, including IL-6 and ALP, did not exhibit a significant difference. As the results have shown, PTX and TP combination have a positive effect on cortical bone angiogenesis, which is evidenced by the high density of microvessels and osteocytes.

These above observations and analyzation of cortical bone parameters are commensurate with our hypothesis that the cortical bone play an important role in the clinical evaluation and prognosis of ORN (Appx, Fig. 7). These results can be applied in ORN surgery treatment. Most importantly, in the treatment of stage II or stage III ORN, the cortical bone can be preserved. Instead of total resection, saucerization treatment with necrosis bone removal can be recommended, leaving the bleeding cortical bone wall for a later bone graft or a reconstruction.

Also, the results regarding the efficacy of PTX + TP as a therapy suggest that this combination can be a choice of medical management of ORN, especially in the early stage. PTX+TP can be prescribed as preventive medicine in post-irradiated patients. According to Delanian *et al*., the dose of 800 mg of PTX and 1000 IU of TP daily for at least 6 months is effective in cessation of progression and promoting bone healing [6]. However, due to the lack of uniformity of this medical treatment, additional well-designed prospective multicenter studies are needed to confirm the optimal effective dosages of PTX and TP.

Conclusion

Through the micro-CT image analysis of this study, cortical bone has proven to play an important role in the clinical evaluation and prognosis of ORN. The combination of PTX and TP has potent antifibrotic properties and improving effect on the cortical bone quality and quantity, resulting in a regression of the disease. The PTX and TP combination can be a reliable treatment protocol or can be used for early ORN prevention in particular susceptible patients. With regard to the jawbones, this study demonstrates that micro-CT is an outstanding instrument for cortical bone assessment and qualification.

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Tables

Table 1. Animal grouping

	Medication	**N***
Irradiation group	Pentoxifylline + Tocopherol (PTX + TP)	10
	Pentoxifylline (PTX)	10 (1)
	Tocopherol (TP)	10 (3)
	Normal saline (NS)	10 (1)
Non-irradiation group (C)		8

*Four animals died after radiation and one animal awoke from anesthesia during irradiation.

Table 2. Comparison of the ABH and CBT on the extracted mandibles between non-irradiation group (C) and irradiation – NS administrated group

		Buccal side	Lingual side
ABH (mm)	C	3.87 ± 0.14	1.75 ± 0.14
	NS	2.72 ± 0.16	0.97 ± 0.20
CBT (mm)	C	0.45 ± 0.07	0.31 ± 0.10
	NS	0.25 ± 0.11	0.17 ± 0.09

The ABH and CBT in the NS group were significantly lower than ABH and CBT in the non-irradiation group.

Table 3. Alveolar bone height of each irradiated group.

	PTX + TP	PTX	TP	NS
HB1	3.29 ± 0.20	3.07 ± 0.09	2.90 ± 0.12	2.72 ± 0.16
HB2	3.59 ± 0.14	3.53 ± 0.19	3.41 ± 0.18	3.29 ± 0.15
HB2 – HB1 (ΔHB)	0.29 ± 0.11*	0.46 ± 0.12	0.51 ± 0.11	0.58 ± 0.11
HL1	1.33 ± 0.14	1.20 ± 0.24	1.03 ± 0.20	0.97 ± 0.20
HL2	1.64 ± 0.18	1.76 ± 0.27	1.66 ± 0.18	1.69 ± 0.22
HL2 – HL1 (ΔHL)	0.31 ± 0.16	0.55 ± 0.14	0.63 ± 0.21	0.72 ± 0.25*

HB1, HB2 (mm): Alveolar bone height (ABH) at the buccal sides of the irradiated jaw and opposite jaw, respectively. HL1, HL2 (mm): ABH at the lingual sides of the irradiated jaw and opposite jaw, respectively. Alveolar bone loss on the buccal side (ΔHB) of the PTX + TP group significantly lower compared to the PTX and NS group. Alveolar bone loss on the lingual side (ΔHL) of the NS group significantly higher compared to the PTX and PTX + TP groups (*$p < 0.05$)

Table 4. Comparison of CBT between irradiation groups

		Buccal side	Lingual side
CBT (mm)	PTX + TP	0.42 ± 0.12*	0.30 ± 0.12*
	PTX	0.32 ± 0.12	0.25 ± 0.06
	TP	0.28 ± 0.11	0.18 ± 0.10
	NS	0.25 ± 0.11	0.17 ± 0.09

The CBT of PTX+TP administrated group was significantly higher than that of other groups (*$p < 0.05$)

Table 5. Cortical bone parameters of the irradiated groups

	PTX + TP	PTX	TP	NS
Ct.BV	33.04 ± 1.88*	30.72 ± 1.32	29.71± 1.55	29.02 ± 1.12
Ct.Th	0.56 ± 0.02	0.54 ± 0.03	0.54 ± 0.03	0.51 ± 0.02*
Ct.S	226.23 ± 11.96*	206.05 ± 13.62	201.75 ± 15.84	207.40 ± 3.87
PoV	1.25 ± 0.20	1.40 ± 0.34	1.19 ± 0.22	1.47 ± 0.29
PoV%	3.53 ± 0.40*	4.52 ± 0.56	4.36 ± 0.69	4.66 ± 0.74

Ct.BV: Cortical bone volume, mm^3; Ct.Th: Cortical bone thickness, mm; Ct.S: Cortical bone surface, mm^2; PoV: Total volume of pore space, mm^3; PoV%: percent of pore space on total cortical bone volume,%. Ct.BV of the PTX+TP group is significantly higher than that of PTX, TP, and NS. CtTh of PTX+TP is significantly higher than that of the NS group. Ct.S of PTX+TP is significantly higher than those of the PTX, TP, and NS groups. The PoV of the four groups did not differ significantly. However, the PoV% for the PTX+TP group is significantly lower than that of PTX and NS, and did not significantly differ from the TP group (*$p < 0.05$)

Table 6. The microvessel density (MVD) obtained from CD31 staining and immunohistochemical staining score of TNF-α, TGF-β1, ALP and IL-10 in the 4 irradiated groups.

	MVD	TNF-α		TGF-β1		IL-6		ALP	
		Score 0-1 (%)	Score 2-3 (%)	Score 0-1 (%)	Score 2-3 (%)	Score 0-1 (%)	Score 2-3 (%)	Score 0-1 (%)	Score 2-3 (%)
PTX + TP	15.4 ± 1.4*	88.2	11.8	78.4	21.6	79.3	20.7	52.2	47.8
PTX	10.2 ± 1.2	80.9	20.1	74.9	25.1	80.0	20.0	58.0	42.0
TP	8.0 ± 2.1	70.6	29.4	60.2	9.8	71.5	28.5	61.1	38.9
NS	5.2 ± 1.0	69.2	30.8	60.5	39.5	72	28	60.0	40.0

0: negative, 1: weak (1%–10% of cells positive), 2: moderate (11%–50% of cells positive), and 3: strong (>50% of cells positive). MVD in the PTX+TP group was significantly higher than those of the PTX, TP and NS groups (*$p < 0.05$). The expression of TNF-α and TGF-β1 was relatively lower in PTX + TP group. The other markers, including IL6 and ALP, did not show significant differences.

Figure legend and Figures

Figure 1. Timeline of animal experiment.

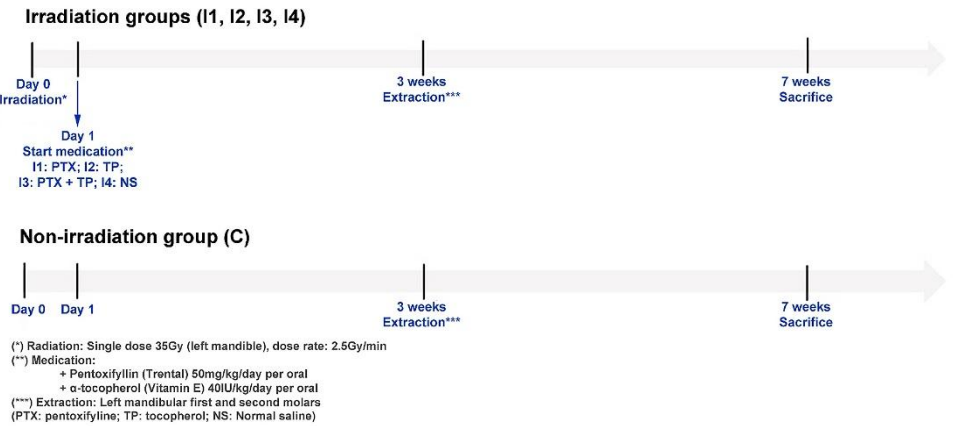
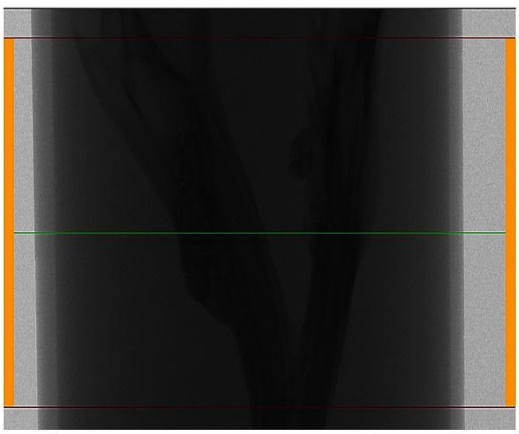
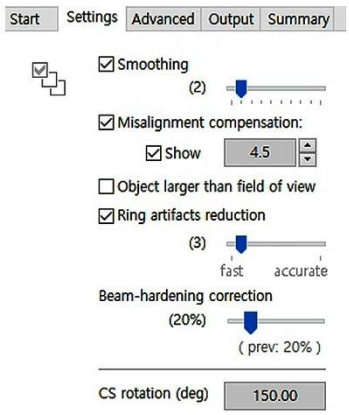


Figure 2. A1, A2. During radiation, the animal was positioned on their right side, the irradiated area was defined as a 1 x 2 cm rectangular on the left mandibular body and the isocenter was located using laser light, **A3**. The external radiation procedure was performed with X-RAD 320 Irradiator (Precision X-ray Inc., North Branford, USA) with high radiation doses (35Gy), **B.** The day after irradiation, the drug delivery according to each defined experimental group was started, **C1, C2.** All rats underwent atraumatic extraction of the first and the second left mandibular molars three weeks after irradiation. **D1, D2, D3.** The animals were sacrificed seven weeks after irradiation and four weeks after extraction with CO2 inhalation. Mandible was isolated, all soft tissue was removed then the mandibular bone was fixed in formalin.

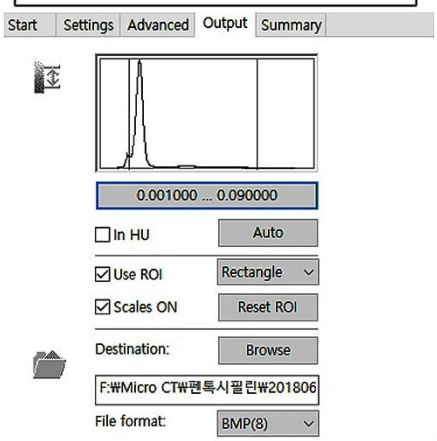
Figure 3. Data sets reconstruction with NRcon software (SkyScan, Aartselaar, Belgium). **A.** Reconstructed region selection, **B.** Setting of reconstruction parameters, **C.** Output setting, **D.** Output region selection.



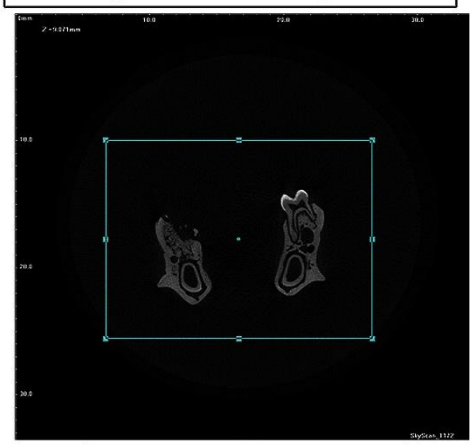
A. Reconstructed region selection



B. Setting of reconstruction parameters



C. Output setting



D. Output region selection

Figure 4. Define of region of interest (ROI) and volume of interest (VOI) using CtAn software (Bruker, Kontich, Belgium). **A, B.** Set the bottom and the top of a fixed data set of 360 slices. The ROI was defined as the whole cortical bone the mandible and was manage semi-manual, **C.** Setting the analyzed values for 3D analysis, **D.** Setting of the task list for data analysis by custom processing.

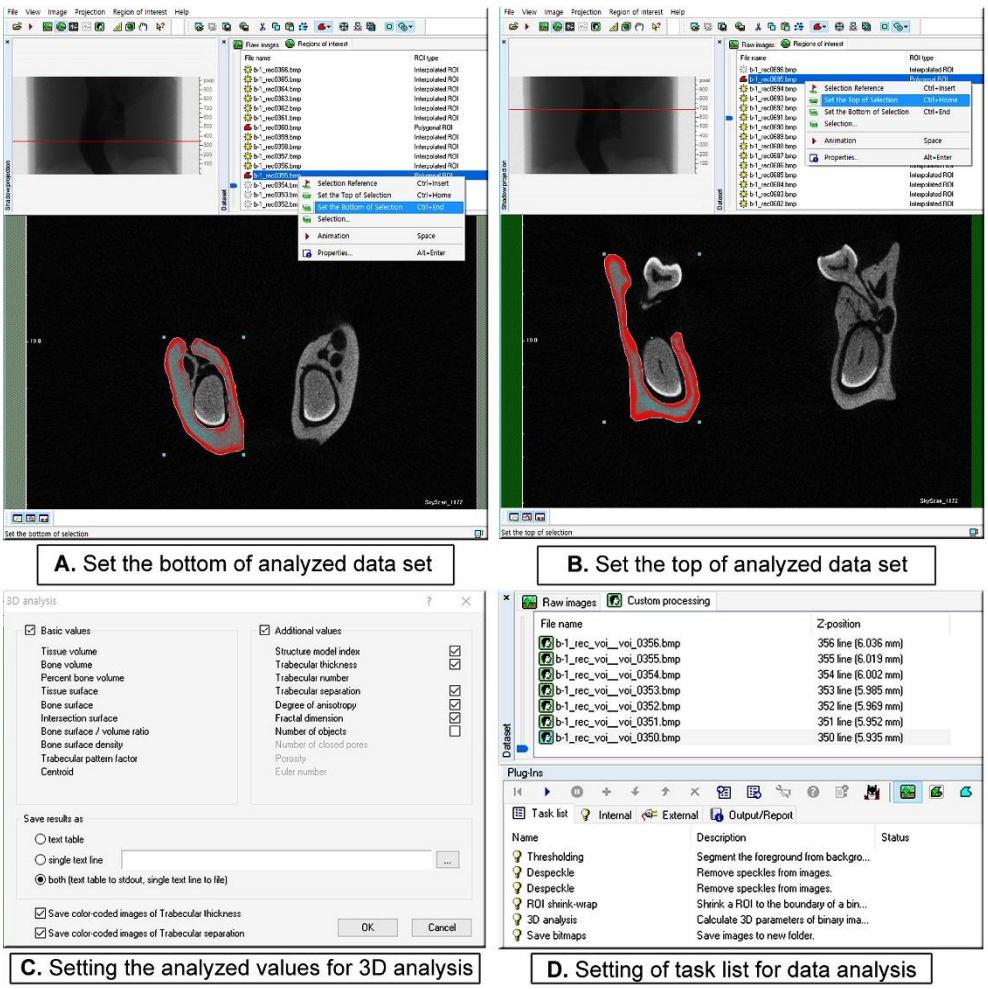


Figure 5. **A.** Selection of one slice using for alveolar bone height and cortical bone thick ness measurement, M1, the first molar, M2, the second molar, M3, the third molar, D, distal root, M, mesial root, IL, intermediate lingual root, IB, intermediate buccal root. Ds, ILs, IBs, Ms: the socket of corresponding tooth roots on the extracted site, **B.** Alveolar bone height measurement method, a is the line drawn tangential to the upper cortical border of the mandibular canal at C and perpendicular to the external surface of the buccal plate b, A point is top of the vestibular crest, A' point is the point of intersection of line draw from A and perpendicular to a, B point is top of the lingual crest, B' point is the point of intersection of line draw from B and perpendicular to a, h1, h2 is the cortical bone height on vestibular and lingual side, respectively, **C.** Cortical bone thickness measurement method, t_1 is cortical bone thickness of buccal wall, t_2 is cortical bone thickness of lingual wall.

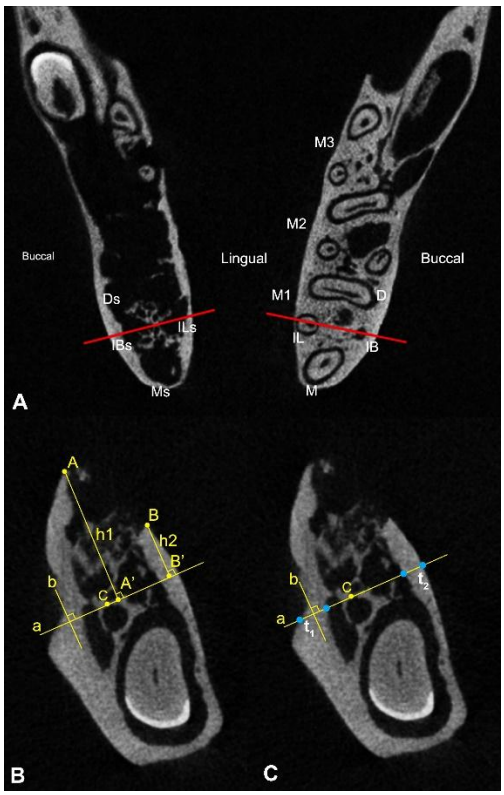


Figure 6. Mandibular changes after irradiation – animal observation and micro-CT findings. A1, Observed skin in mandibular area, A2, Observed post-extraction wound healing in left mandible, A3, Bone remodeling observed on micro-CT. **A**, Non-irradiation group, B, PTX administrated group, **C**, TP administrated group, **D**, PTX + TP administrated group, **E**, Normal saline administrated group. Black arrow, depilation of the skin and oral mucosa wound and necrosis lesion, Blue arrow, bone healing and new bone formation, Yellow arrow, socket bone failed to heal, presence of necrosis bone, the calcification of incisor pulp chamber.

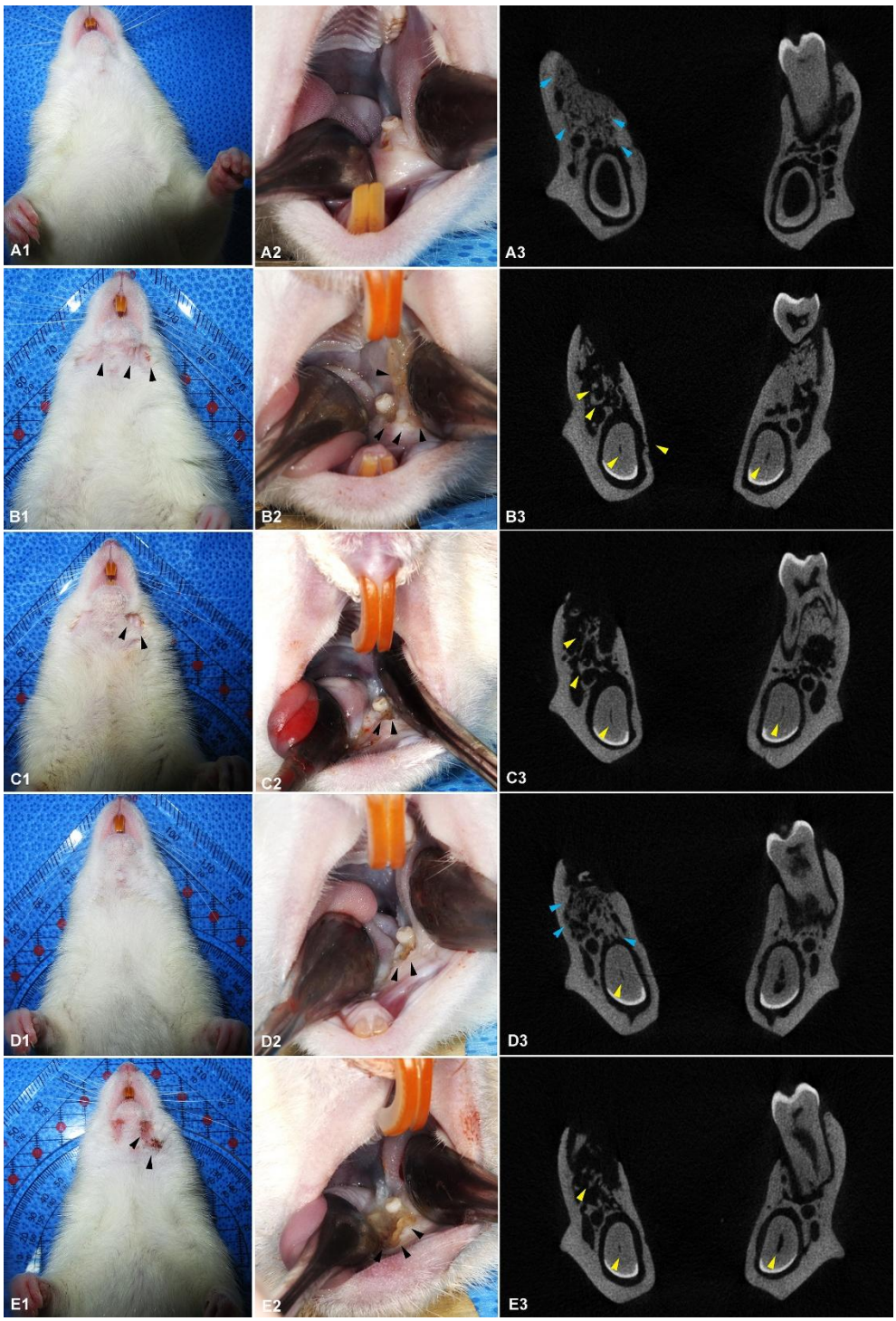

A1
A2
A3
B1
B2
B3
C1
C2
C3
D1
D2
D3
E1
E2
E3

Figure 7. Ct.BV (Cortical bone volume) of PTX + TP group was significantly bigger than which of pentoxifylline, tocopherol and normal saline groups (*$p < 0.05$)

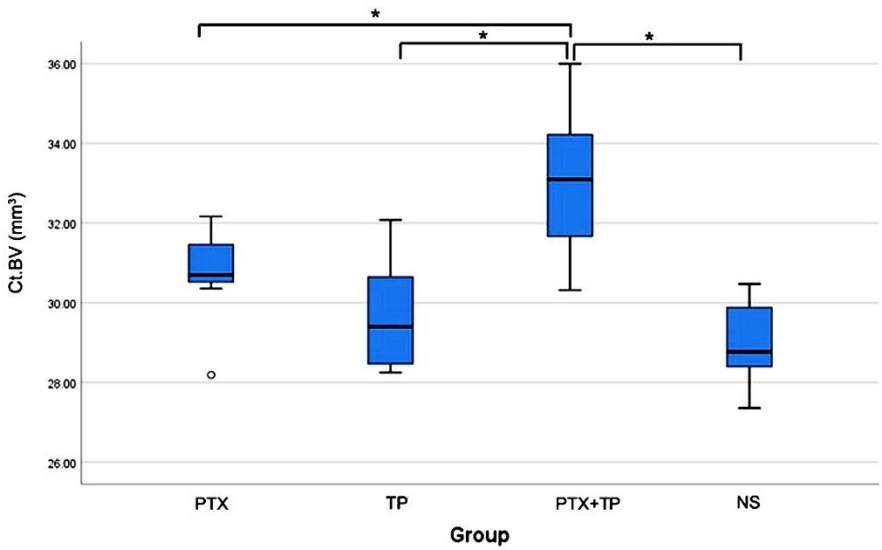


Figure 8. Ct.S (Cortical bone surface) of PTX + TP is significantly larger than which in PTX, TP and NS (*$p < 0.05$)

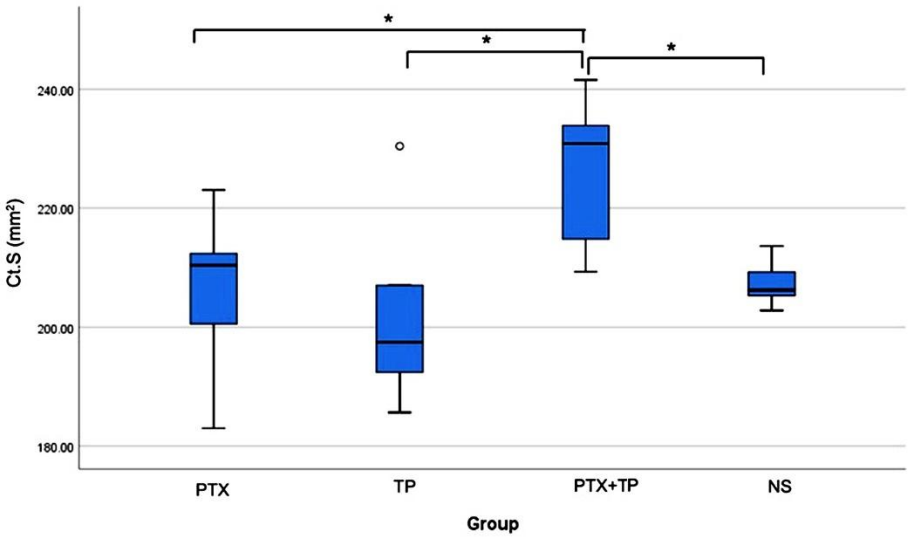


Figure 9. Ct.Th (Cortical bone thickness) of PTX + TP group was significantly larger than which in NS group (*$p < 0.05$)

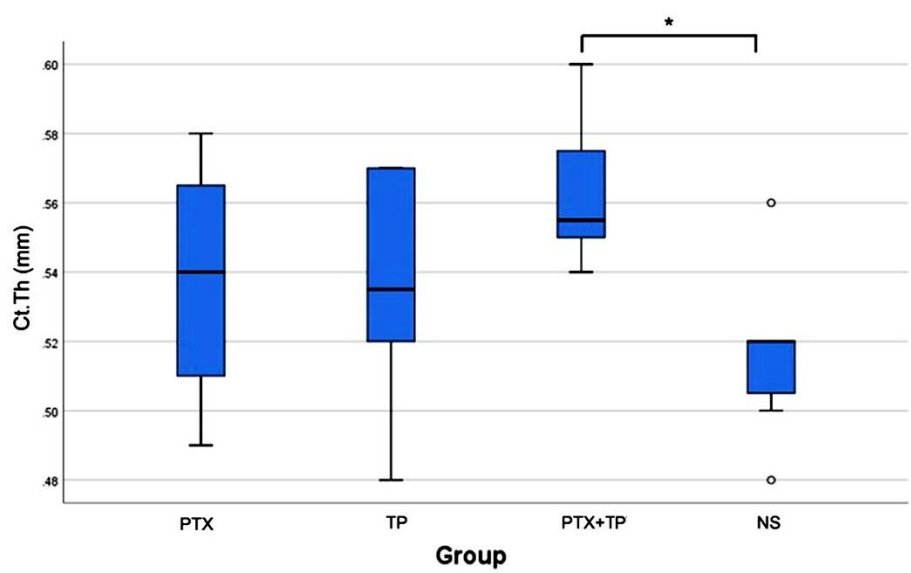


Figure 10. The PoV (Total volume of porosity) of four groups did not differ significantly (*$p < 0.05$)

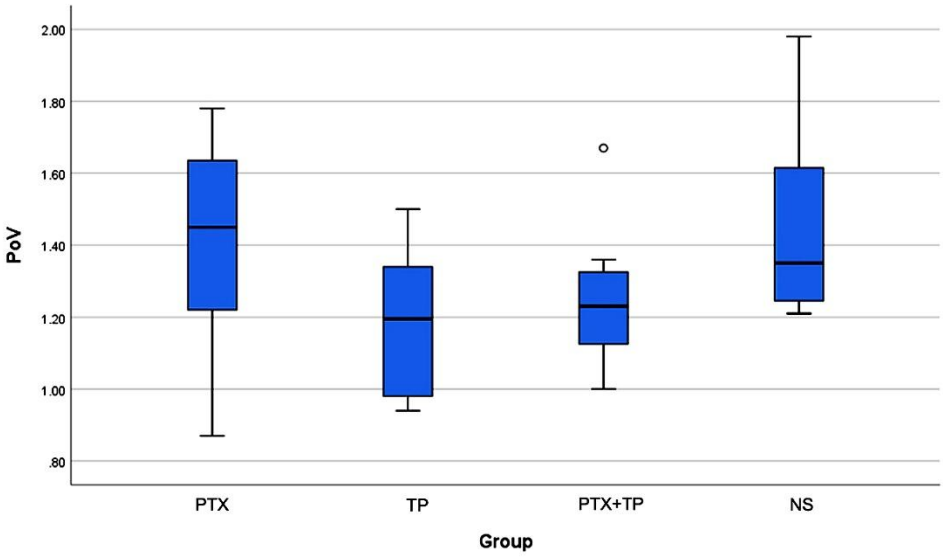

2.00
1.80
1.60
1.40
1.20
1.00
.80
PoV
PTX
TP
PTX+TP
NS
Group

Figure 11. PoV% in PTX + TP group is significantly higher than which in pentoxifylline and normal saline, and did not significantly differ to which of TP group ($p < 0.05$).

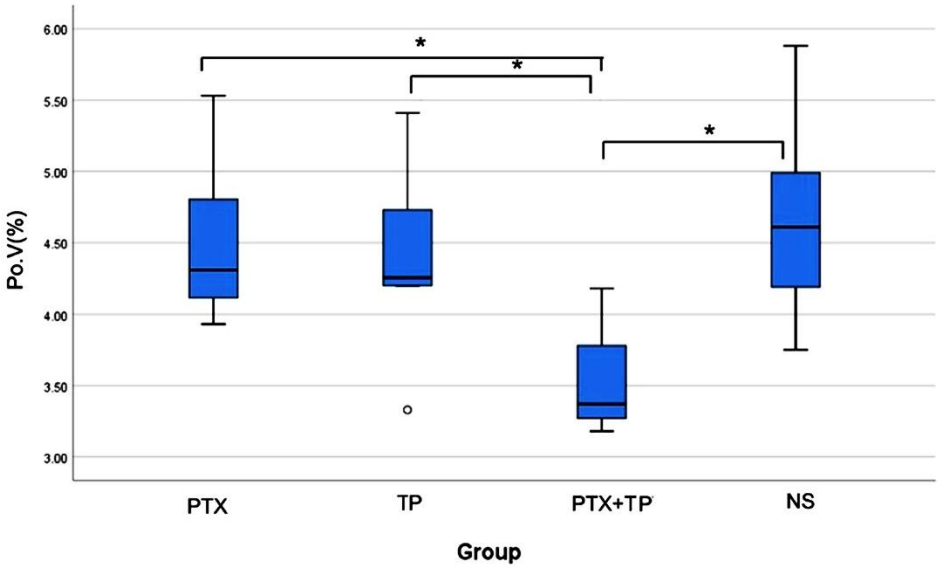


Figure 12. Three – dimension reconstruction of the cortical bone in irradiated groups: PTX, TP, PTX + TP.

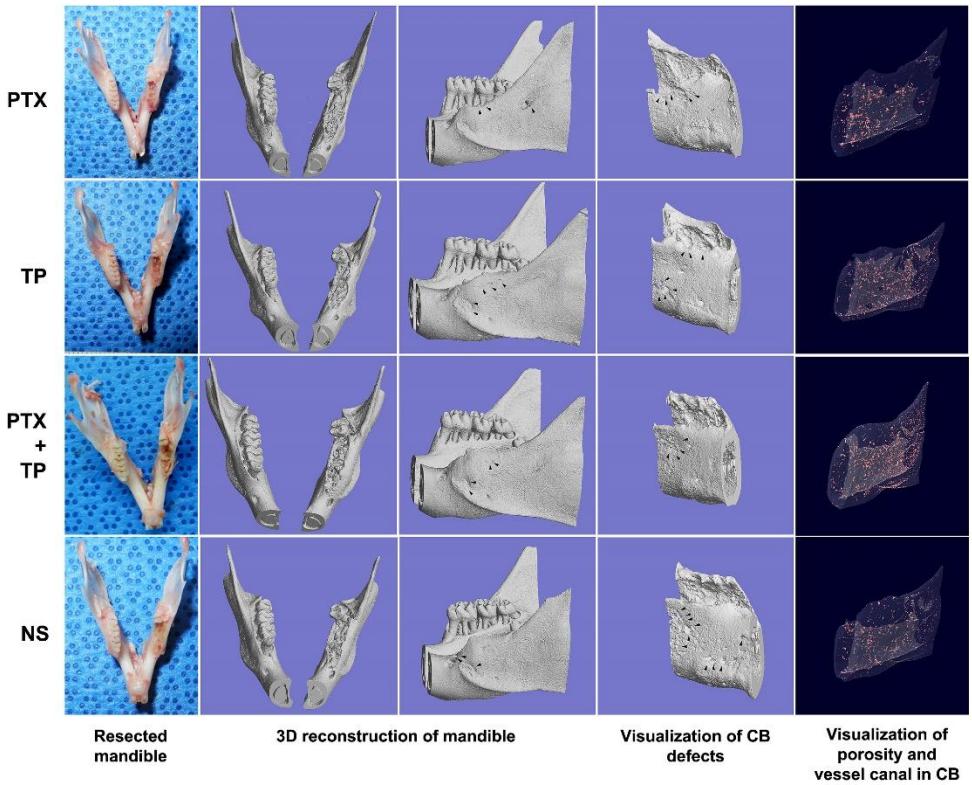


Figure 13. Three-dimension reconstructed images of mandible in irradiated group. Images show the cortical bone thickness difference in PTX + TP, PTX, TP and NS groups.

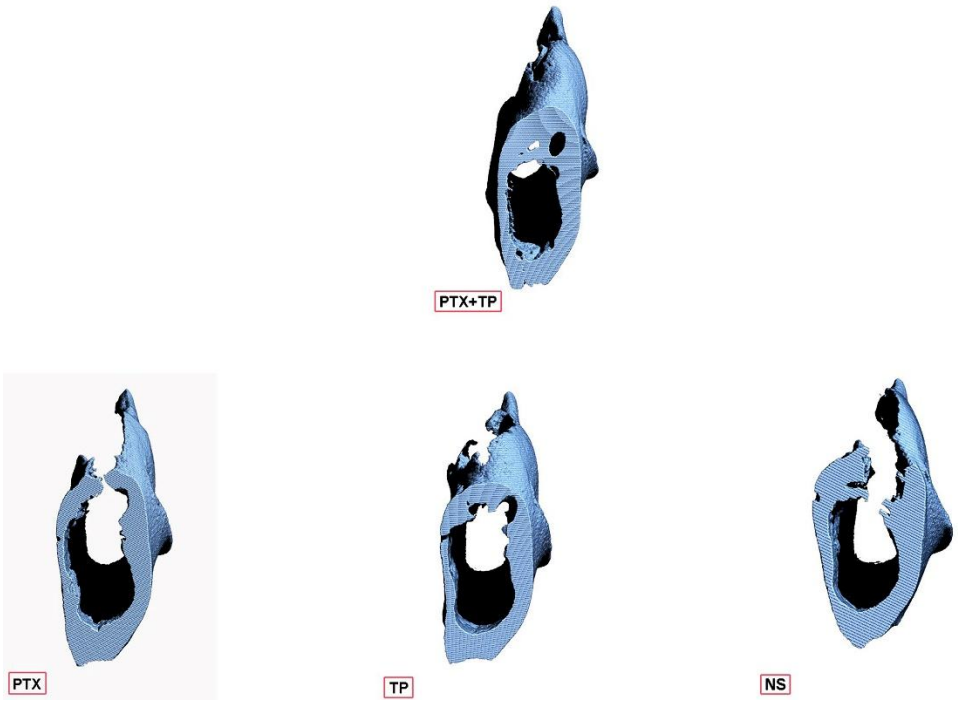


Figure 14. Three-dimension reconstructed images of mandible in irradiated group. Images show the difference in lingual cortical bone wall defect in PTX + TP, PTX, TP and NS groups. (change all the grouping according to this figure)

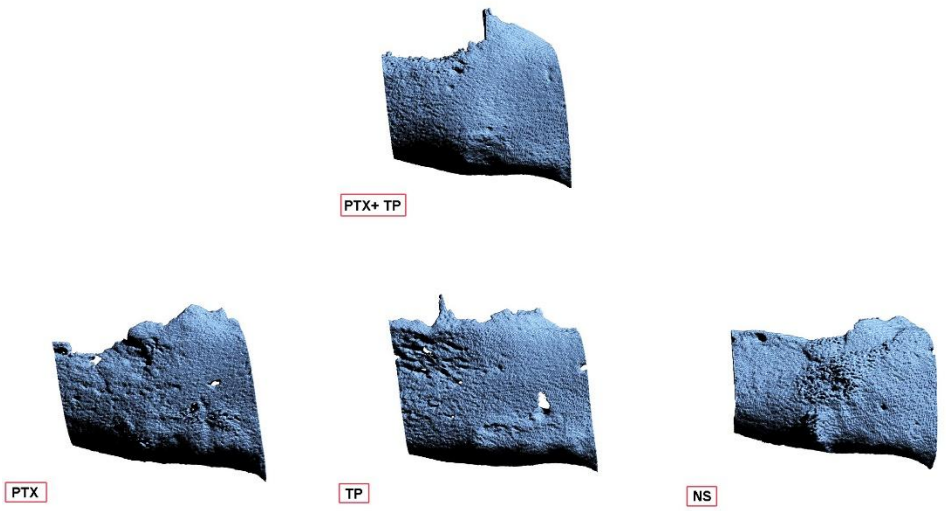


Figure 15. H&E staining in irradiated groups. **A**, PTX group, **B**, TP group, **C**, PTX + TP group, **D**, NS group. A1, H&E staining with original magnification, x400, A2, Magnified image of the selected region from A1, x100, Red arrow, viable blood vessels within Haversian canals, Yellow arrow, nonviable blood vessels within Haversian canals.

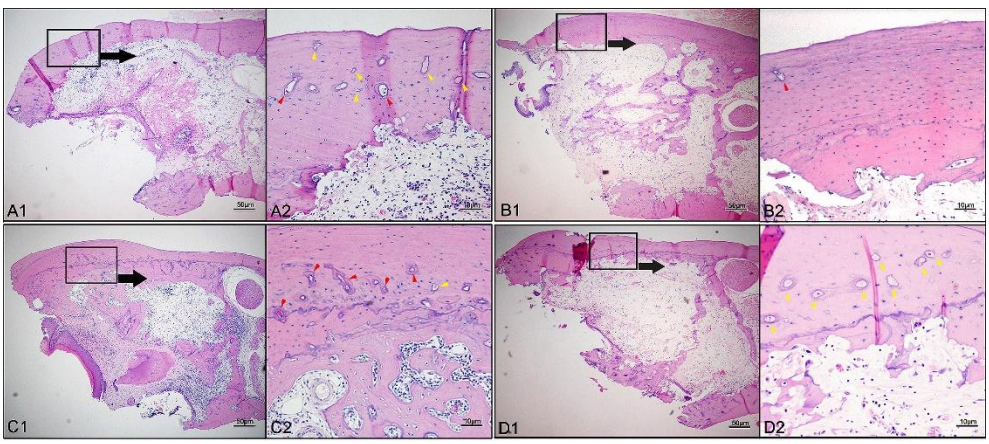


Figure 16. CD31 (PECAM) staining in irradiated groups. **A**, PTX group, **B**, TP group, **C**, PTX + TP group, **D**, NS group. A1, H&E staining with original magnification, x400, A2, Magnified image of the selected region from A1, x100. The arrows making the microvessels in cortical bone.

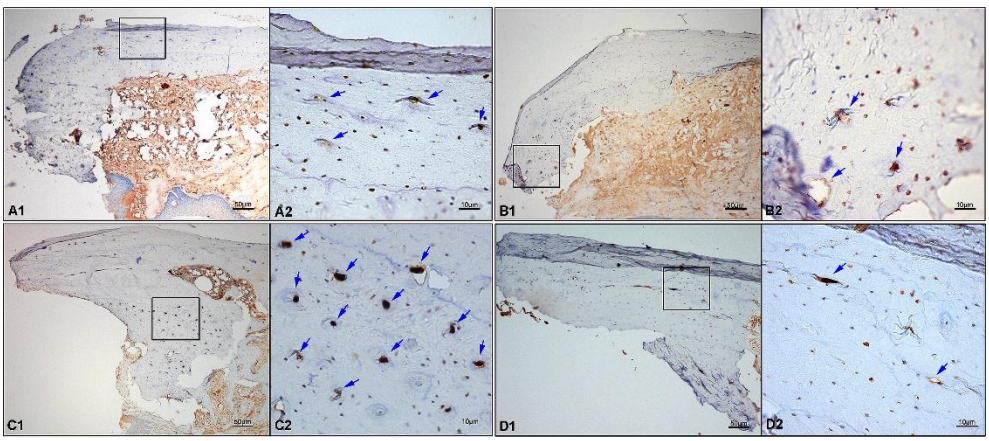
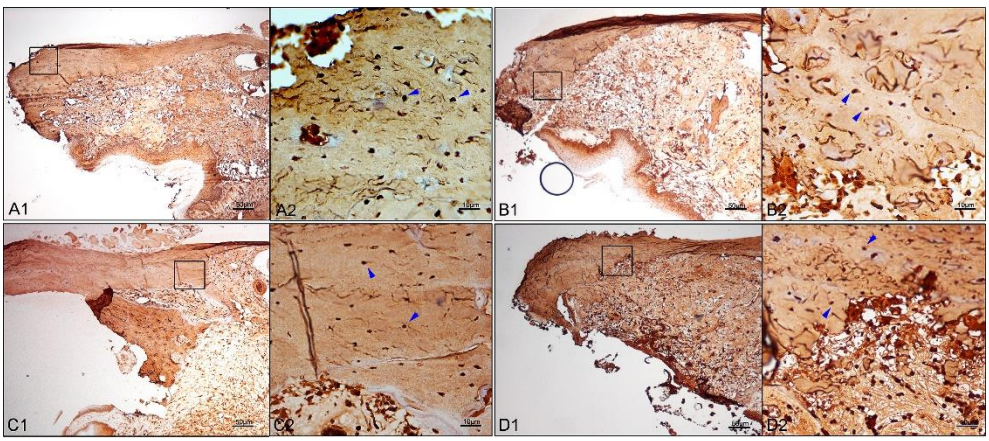
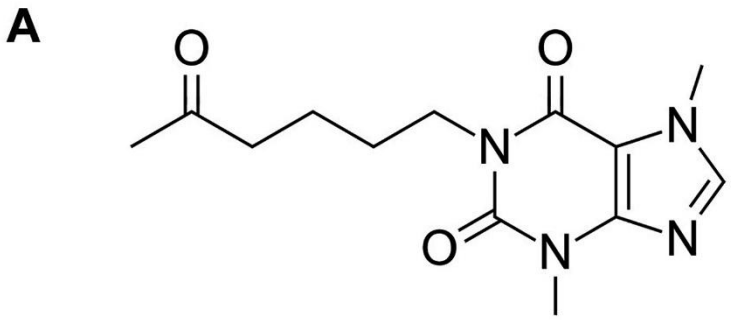


Figure 17. Osteocalcin (OC) staining in irradiated groups. **A**, PTX group, **B**, TP group, **C**, PTX + TP group, **D**, NS group. A1, H&E staining with original magnification, x400, A2, Magnified image of the selected region from A1, x100. The arrows making the osteocytes in cortical bone.

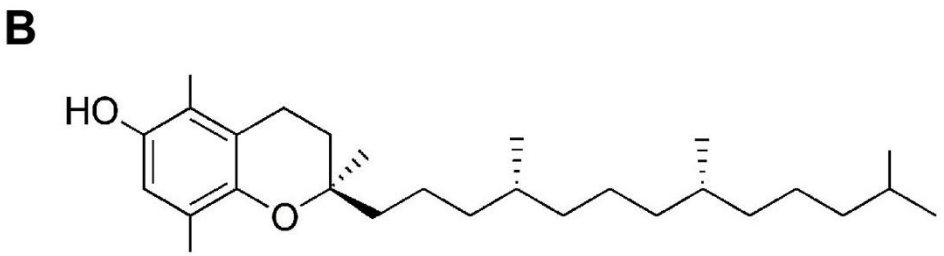


Appendix

Figure 1. **A.** Structure of pentoxifylline **B.** Structure of α-tocopherol (Vitamin E)



Pentoxifylline: 1 - (5 - oxohexyl) - 3,7 - dimethylxanthine



α-Tocopherol (Vitamin E)

Figure 2. Clinical and panoramic view of BRONJ patient. **A.** Pre-operative panoramic view, **B-D.** Pre-operative CT views in axial, sagittal and coronal plans, **E.** Intraoral fistula of the BRONJ lesion, **F,G.** Necrotic cortical and cancellous bone were removed during surgery, **H.** Post-operative panoramic view, **I.** Post-operative intraoral view. Yellow arrows making the extensive bone destruction in cortical and cancellous bone**.**

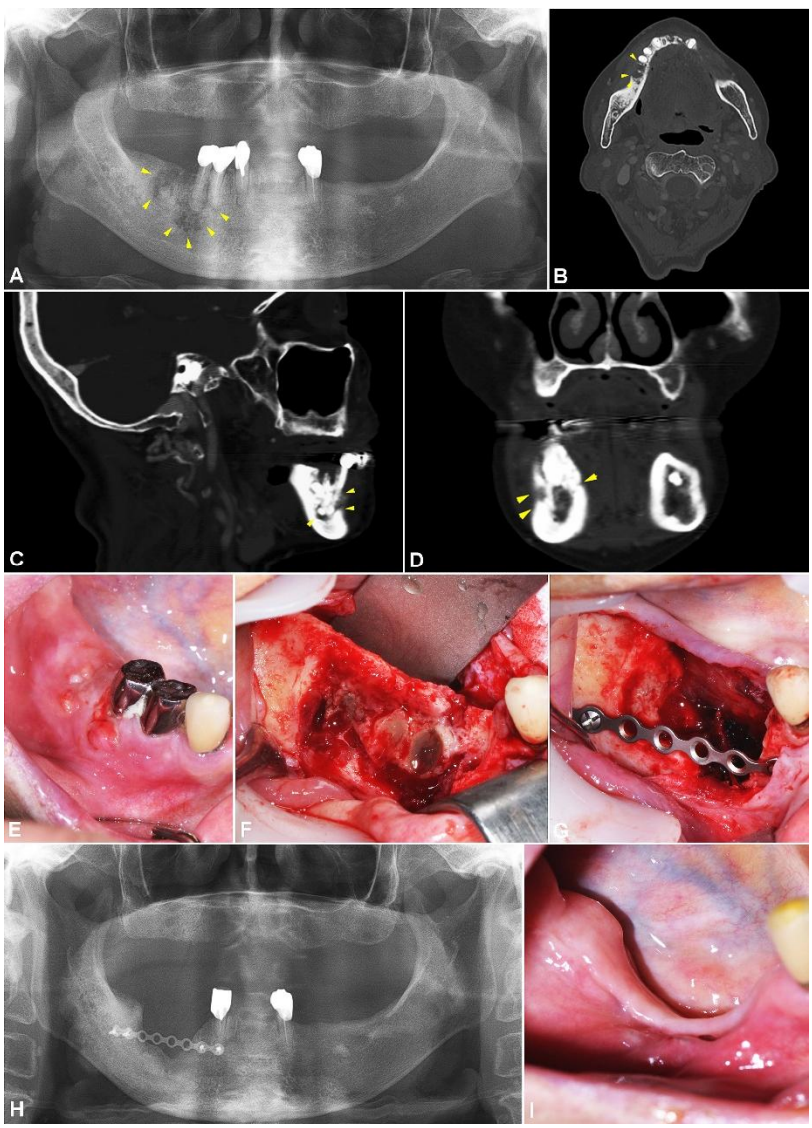


Figure 3. Clinical and panoramic view of ORN patient with complex defects. **A-C.** Panoramic, axial and coronal view of ORN defects in patient's first examination (right mandible), **D-F.** Panoramic, axial and coronal view of ORN defects on the opposite side 7-year after the first ORN diagnosis, **G-I.** Intraoral view of recurrent ORN on the right mandible which required bridge removal and tooth extraction. Yellow arrows making the complex defects in left and right side of the mandible.

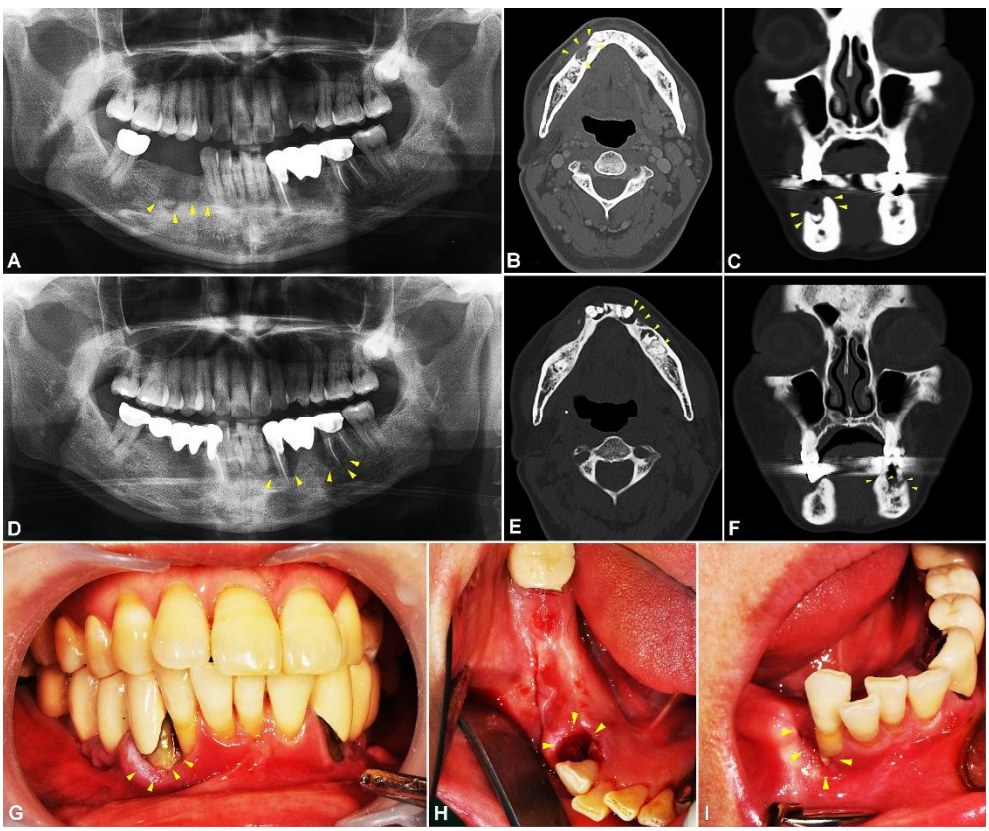


Figure 4. Clinical and panoramic view of progressive mandibular ORN patient. **A**. Pre-operative panoramic view, **B,C**. Pre-operative axial and coronal CT views, **D**. Intraoral view of the ORN lesion region, **E-G**. Removal of necrotic cancellous and a part of cortical bone during saucerization surgery. A part of cortical bone wall with vital sign was preserved. Yellow arrows making the cancellous bone destruction and the remained cortical bone can be observed on CT views.

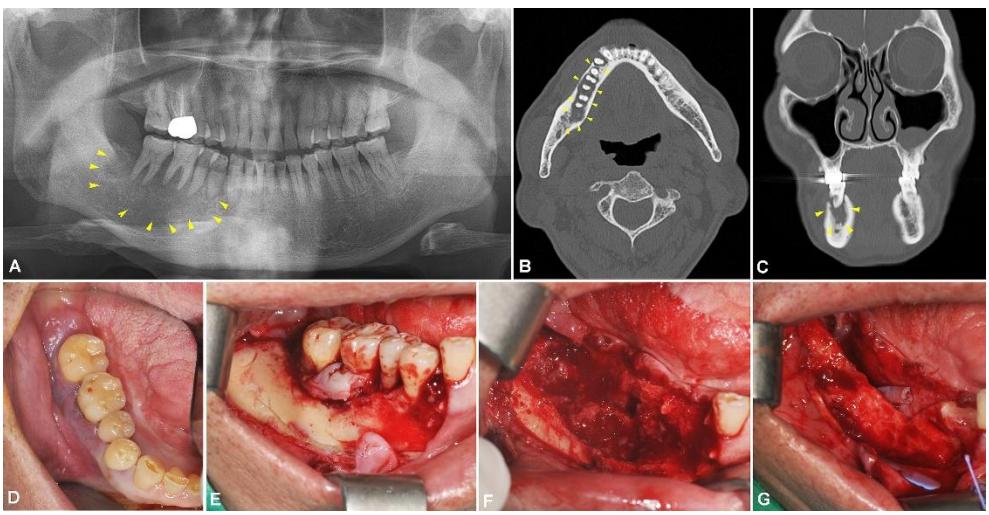


Figure 5. Pathophysiology of Osteoradionecrosis according to Marx

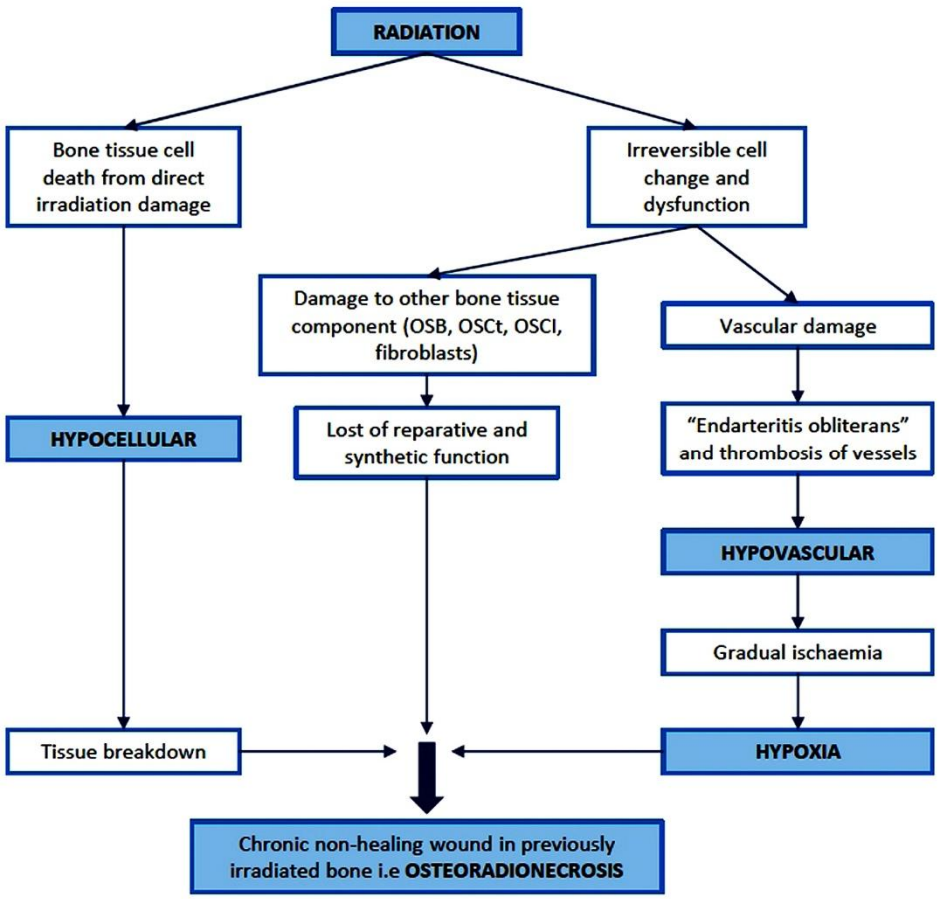


Figure 6. Radiation-induced fibroatrophic theory by Delanian *et al*.

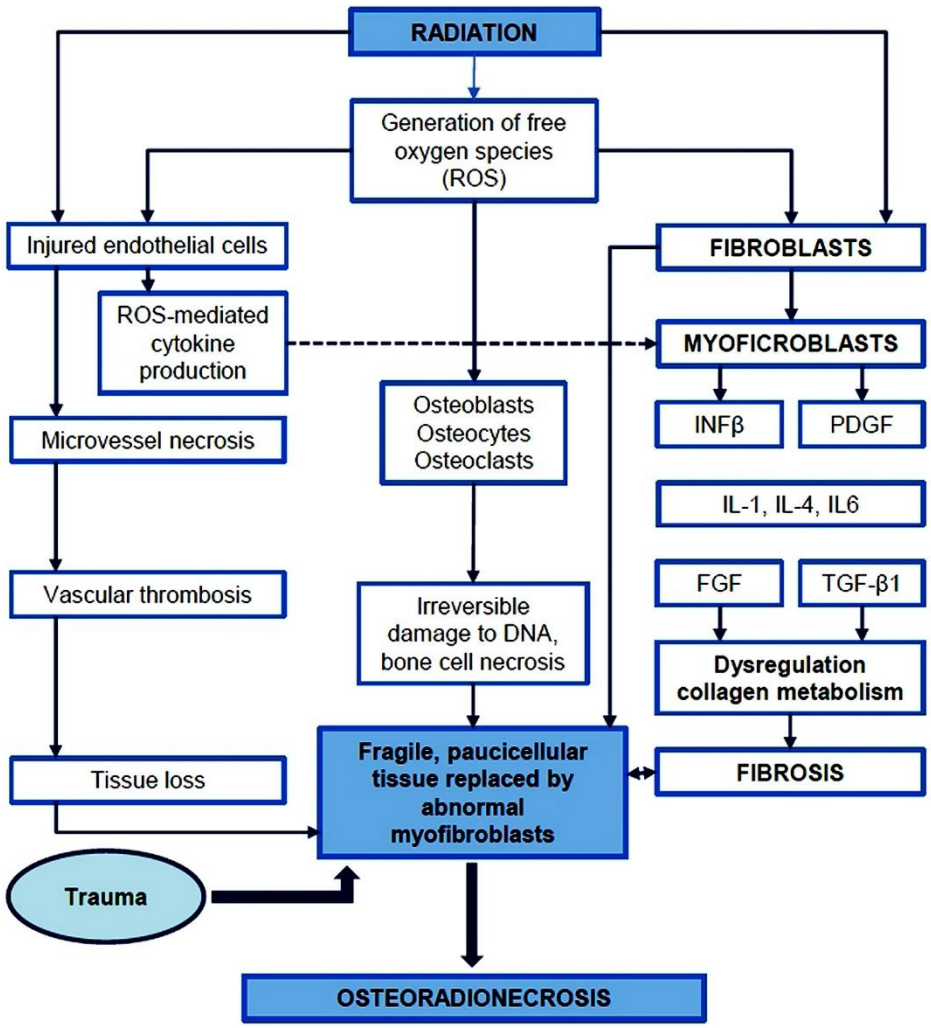
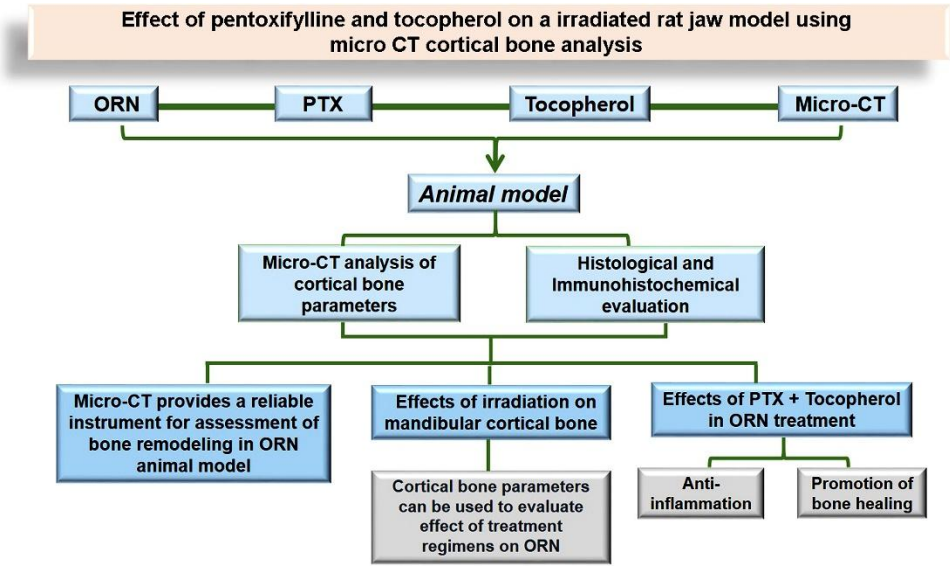


Figure 7. Flow chart of study design and results.



-국문 초록-

펜톡시필린과 토코페롤의 방사선 조사 백서 악골에서의 피질골량과 골질 증진에 대한 미세 단층촬영 연구

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연구 배경 및 연구 목적

두경부 악성 종양의 치료 있어 방사선 치료는 일차적으로 근치적 치료 요법, 수술적 치료의 보조적 요법, 항암 치료와 함께 하는 병용 요법 또는 절제 불가능한 경우 고식적 치료 요법으로 사용되고 있다. 최근에는 방사선 치료 기술이 발전함에 따라, 예상하지 못한 합병증들이 국소화되고 있다. 그러나, 여전히 환자들은 점막, 하방의 턱뼈, 타액선의 방사선에 대한 반응으로 고통을 받고 있으며, 그 중에 가장 특이할 만한 합병증은 하악의 방사선 골괴사증 (osteoradionecrosis, ORN)이다. 펜톡시필린 (pentoxifylline)과 토코페롤 (tocopherol)은 강력한 섬유화 방지 효과를 가지고 있으며, 이는 만성 섬유화를 감소시키고 ORN 에서 골치유를 촉진시키는 것으로 알려져 왔다. 펜톡시필린과 토코페롤의

병용 요법은 수술적 치료에 보조적으로 또는 ORN 의 비침습적 치료 방법으로 사용될 수 있다. 본 연구에서는 새로운 micro-CT 패러다임을 사용하여 ORN 동물 모델에 펜톡시필린과 토코페롤을 투여하여 약제의 효과를 피질골 분석을 통해 평가하고, 이를 바탕으로 ORN 의 병태생리 및 최근의 치료법에 대하여 논의할 것이다.

연구 방법

8 주령의 평균 몸무게 369.6g 인 수컷 Sprague-Dawley 백서 (오리엔트바이오, 성남, 한국) 48 마리를 실험에 사용하였다. 백서는 무작위로 실험군으로 방사선 조사군 (40 마리)과 대조군으로 방사선 조사를 받지 않은 군 (8 마리)으로 나누었다. 방사선 조사군은 투여한 약물에 따라서 PTX (펜톡시필린, pentoxifylline), TP (토코페롤, tocopherol), PTX+TP (펜톡시필린과 토코페롤, pentoxifylline and tocopherol)와 NS (생리식염수, normal saline)로 나누었다. 약제는 실험 기간 종료시까지 투여하였다. 방사선 조사 3 주 후에, 하악 구치의 발치를 진행하였다. 실험 동물은 방사선 조사 7 주, 발치 4 주 후에 희생하였다. 채취한 하악골은 micro-CT 와 조직학적 평가를 통해 분석하였다.

연구 결과

1. Micro-CT 분석

방사선 조사군 중에 NS 군과 대조군 (C)에서 치조골 높이 (alveolar bone height, ABH)와 피질골 두께 (cortical bone thickness, CBT)를 비교하였다. ABH 와 CBT 는 NS 군에서 방사선 비조사군 (C)에 비하여 낮게 나타났다. 방사선 조사 실험군에서 투여한 약제에 따라 ABH 와 CBT 를 비교하였다. 펜톡시필린과 토코페롤을 함께 투여한 군에서 ABH 와 CBT 는 다른 실험군에 비하여 유의미하게 높게 나타났다. PTX+TP 군에서 ABH 와 CBT 는 유의미한 차이를 나타내지는 않았다.

PTX 군에서 CBT 는 NS 군에 비하여 유의미하게 높게 나타났다. ($p < 0.05$).

모든 방사선 조사군에서 설측의 치조골 소실 (ΔHL)이 협측의 치조골 소실 (ΔHB)에 비하여 유의미하게 높게 나타났다. 협측에서, PTX+TP 를 사용한 그룹에서 PTX 그룹과 NS 그룹에 비하여 ΔHB 가 유의미하게 낮게 나타났다. 설측에서는 NS 그룹의 ΔHL 이 PTX + TP 그룹의 ΔHL 에 비하여 유의미하게 높게 나타났다 ($p < 0.05$).

방사선 조사군 중 PTX + TP 군에서 피질골 부피 (cortical bone volume, Ct.BV)와 총피질골 표면적 (Ct.S)이 PTX, TP, NS 군에 비하여 유의미하게 높게 나타났다. 총기공 부피 (total pore volume, Pov)은 약제에 따른 차이를 보이지는 않았다. 총기공 부피율 (total pore rate, PoV%)는 PTX + TP 군에서 PTX 군이나 NS 군에 비해서 유의미하게 나타났으며 ($p < 0.05$), TP 군과는 유의미한 차이를 보이지는 않았다.

2. 제 3 차원 (3D) 재구성된 이미지 평가

3D 재구성된 이미지에서 피질골 결손 부위를 평가하였다. 모든 방사선 조사 군에서 협측골의 결손된 상태에 비하여 설측골의 결손된 상태가 심하게 나타났다. PTX+TP 군의 결손부 상태는 PTX, TP, NS 군에 비하여 양호하였다. 발치와에서 피질골 결손부와 해면골 결손부 사이의 상관관계를 평가하기 위해 3D 이미지를 사용하였다. 방사선 비조사군에서 골치유가 일어났으며, 발치와는 새롭게 형성된 해면골로 채워지고, 건전한 피질골 벽으로 둘러 쌓여 있는 소견을 보여주었다. 방사선 조사군에서 치유되지 않은 발치와와 골괴사가 관찰되었다. 그리고 잔존한 피질골과 해면골의 부피가 불충분함을 보여주었다.

3. 조직 학적 및 면역 조직 화학적 분석

방사선 조사군에서 골세포가 없는 골소강이 관찰되었는데, 특히 NS 군에서 그러하였다. PTX + TP 군에서 하버스관 (haversian canal)에서

살아있는 혈관이 관찰되었으며 PTX 군에서도 적은 양으로 관찰되었다. TP 와 NS 군에서는 하버스관에서 실활된 혈관이 우세하였다.

CD31 (PECAM) 염색을 사용하여 미세 혈관 밀도 (microvessel density, MVD)를 분석하였다. PTX + TP 군에서 MVD 는 PTX, TP, NS 군에 비하여 MVD 가 유의미하게 높게 나타났다 ($p < 0.05$). osteocalcin (OC) 염색은 피질골 내의 골세포를 평가하기 위해 사용하였다. NS 군에서 OC+ 골세포의 수가 다른 실험군에 비하여 유의미하게 낮게 나타났다 ($p < 0.05$). PTX +TP, PTX, TP 군 간에서는 유의미한 차이를 나타내지는 않았다. TNF-α 와 TGF-β1 의 발현은 PTX+TP 군에서 상대적으로 낮게 나타났다. IL-6, ALP 와 같은 다른 마커들에서는 유의미한 차이를 나타내지 않았다.

결론

결과적으로, 피질골은 ORN 의 임상적 평가 및 예후에 있어 중요한 역할을 하는 것으로 나타났다. 부가적으로, PTX 와 TP 는 병용하여 사용하였을 때 강력한 섬유화 억제 작용이 있어 ORN 의 치료에 긍정적인 효과를 가지고 있으며, 질환 진행을 후퇴시킬 수 있다. 악골에서 micro-CT 는 피질골 분석에서 신뢰 가능한 도구이며, 다른 골구조물 지표의 정량 분석을 강화하는 데 도움을 줄 것이다.

주요어 : 방사선 골괴사증, 펜톡시필린, 토코페롤, 미세 단층 촬영 (micro-CT), 피질골.

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