Effects of Local and Whole Body Irradiation on the Appearance of Osteoblasts During Wound Healing in Tooth Extraction Sockets in Rats

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Radiation/Osteoblasts/Bone formation/Tooth extraction/Wound healing.

Irradiation before tooth extraction delays wound healing in the alveolar socket. This study examined the influences of local and whole body irradiation before tooth extraction on appearance of osteoblasts in the alveolar bone of rat maxillary first molars because bone formation is observed at the initial phase of wound healing. Several osteoblasts were generated 3 days after tooth extraction, and the number of cells increased day by day. Morphological studies showed there were little differences between local irradiation and non-irradiated controls. In contrast, the extraction wound in the whole body irradiation group showed delayed healing, and there was poor granulation tissue and very few osteoblasts at the bottom of the socket. An ultrastructural study showed that the osteoblasts in the extraction socket of whole body irradiation rats were smaller, and had poorly developed organelles. Injection of bone marrow cells to whole body-irradiated animals immediately after tooth extraction partially restored the number of osteoblasts. New periosteal bone formations outside of sockets showed little delay in the whole body irradiation group. These findings suggest that bone formation in the wound healing of extraction socket requires bone marrow cells from hematopoietic organs such as the bone marrow as well as local sources around the alveolar socket, during the initial phase of wound healing.

INTRODUCTION

Radiotherapy is often a useful treatment for carcinomas of the oral cavity in combination with surgery.² However, intact tissues within the radiation field may experience irrecoverable damage depending on the quantity of ionizing radiation employed. The adverse sequelae of radiotherapy arise from acute injuries that result from the reproductive death of rapidly dividing cells and from chronic injuries that are caused by damage to the slowly dividing parenchymal cells and the microvasculature.²,³) The necrotizing effects of radiation on bone tissue are known as osteoradionecrosis. Osteonecrosis is one of the more serious and unpredictable complications of head and neck irradiation for cancer. Most bone problems first appear 3–12 months after irradiation, but some risk remains for many years thereafter, especially if the patient undergoes dental extraction at some time in the future.¹,³,⁴) The overall risk of osteoradionecrosis in edentulous patients is less than that in dentulous patients. Therefore, there is general agreement that teeth with doubtful prognosis should be removed prior to irradiation.⁵) The healing events in the tooth extraction socket culminate in the formation of woven bone, which ultimately remodels, thus resulting in the restoration of the defect.⁶) The healing in the early weeks following tooth extraction has been histologically studied in the rat.⁷) However, the origin of these osteogenetic tissues which cause infilling of the extraction socket still remain controversial, even though the osteoblasts may originate from the residual periodontal ligament, periosteum, bone marrow, or blood vessel-associated pericytes.⁸) Alveolar healing is delayed even in animals that are irradiated 2 weeks prior to tooth extraction in comparison to the healing in animals that are irradiated immediately after extraction.⁹) A decreased number of osteoblasts, osteocytes and hypocellular bone tissue following radiotherapy have been histologically demonstrated in the tooth extraction socket. In addition, the osseous structure is disturbed.⁹) However, few studies have been made on the effect on

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This study chronologically counted the number of osteoblasts with alkaline phosphatase activity and observed the histopathological features characteristic of the irradiated osteoblasts that differentiated during wound healing of extraction sockets to examine influence of local or whole body irradiation before tooth extraction on the appearance of osteoblasts in the alveolar extraction socket. Furthermore, the appearance of osteoblasts in healing alveolar sockets that had received whole body radiation before extraction was examined after transplantation of bone marrow cells from intact animals. The present results suggest that osteoblasts in the healing alveolar socket are related to hematopoietic organs via the circulation in the new bone formation.

**MATERIALS AND METHODS**

The animals were maintained throughout the experiments following the Principles of Laboratory Animal Care established by the NIH. The animal experimentation described in this study was previously approved by the Institutional Committee for Animal Experimentation.

**Animals and experimental design**

Eighty-five male Wistar rats, 100 g body weight, were used in this study. The animals were divided into four groups: a non-irradiation group (N = 25), a local irradiation group (N = 25), a whole body irradiation group (N = 25), and a transplantation group (N = 10). In the non-irradiation group, anesthesia was only administered as a control. The local irradiation group was anesthetized with an injection of pentobarbital sodium (Nembutal, Abbott Laboratories, Abbott Park, IL, USA). They were fixed in the lateral position to turn the left teeth upside and the field was set at 10 × 10 mm with lead blocks used in maxillary left first molar tooth to ensure as constant an exposure dose as possible. The animals in the whole body irradiation group and transplantation group were irradiated in cages. These three groups were irradiated at 8 Gy (1 fraction, dosage rate 0.65 mGy/min, FSD 80 cm) using a 60Co gamma ray unit (Toshiba, Tokyo, Japan).

The maxillary left upper molar was extracted 7 days after irradiation. Five rats in each group were killed by an overdose of anesthetics 5 days after tooth extraction (Fig. 1). Bone marrow from intact rats was transplanted into whole body-irradiated rats in the bone marrow transplanted group. The femurs of intact rats were cut off at both extremities with scissors, and a bone marrow cell suspension was prepared by rinsing by the femoral cavity with a minimum essential medium. The cell suspension was diluted to 1 × 10^7 cells/mL. After tooth extraction, the bone marrow cells were immediately injected into the abdominal cavity in order to simply put all these cells in body. Animals were killed 3 and 5 days after tooth extraction and the transplantation of bone marrow cells.

**Tissue preparation and light microscopic observation**

The removed maxillae were fixed in 4% paraformaldehyde buffered with 0.1 M sodium cacodylate (pH 7.4) for 24 h at 4°C, decalcified in 5% ethylenediaminetetraacetic acid (EDTA) for 14 days at 4°C and then were embedded in paraffin according to a routine method. The sections were cut at the level of the distal socket of the first molar tooth in a bucco-lingual plane with a microtome. These sections were stained with hematoxylin and eosin (HE) for histology and azan to observe new bone formation. Alkaline phosphatase (ALP) activity in differentiated osteoblasts was also detected by enzyme histochemistry using a simultaneous azo dye technique (Burstone’s method). The appearance of osteoblasts and new bone formation during the wound healing of tooth extraction socket were evaluated on the buccal side of the periosteal surface of the alveolar bone (Fig. 2). The number of ALP-positive osteoblasts on newly formed...
bone was counted within a square (500 μm × 500 μm) in the bottom of distal sockets. In addition, the osteoblasts between the upper alveolar bone and the periosteum were counted in the same square field.

Transmission electron microscopic observation

Five days after tooth extraction, maxillae that were removed in each group were immersed in a mixture of 4% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.4), and were post-fixed with OsO4. All samples were dehydrated at graded concentrations of ethanol and embedded in an epoxy resin. (TAAB, Aldermaston, England). Ultrathin sections were cut with a diamond knife on a MT5000 ultramicrotome (Dupont, Delaware, USA) and were double-stained with uranyl acetate and lead citrate. These sections were examined with a JEM-100CX transmission electron microscope (JEOL, Tokyo, Japan).

RESULTS

Extraction wound healing was clearly observed by histological examinations from day 1 to 5 after extraction in the non-irradiation group. Granulation tissue accompanying with many capillaries was found at the bottom of the socket at day 2. The socket was filled with granulation tissue, which was dense with fibroblasts on day 3. Some osteoblasts were seen in the granulation tissue at the bottom of the extraction socket (Fig. 3a). The histopathological findings of wound healing in the local irradiation group showed a tendency similar to that in the non-irradiation group, although there were slightly fewer fibroblasts and osteoblasts than those in the non-irradiation group (Fig. 3b). On the other hand, the extraction wound in the whole body irradiation group showed delayed healing (Fig. 3c). There were a number of red blood cells and a small amount of granulation tissues with poor fibers on the socket floor. The blood clot was seen in the area about one half distance from the bottom of the socket, and there was poor granulation tissue and very few osteoblasts at the bottom of the socket. However, granulation tissue occupied the area of the healing socket, and a substantial number of osteoblasts were aligned on the surface of

![Fig. 3. Histopathological features of the bottom of the socket. Three days after tooth extraction. (a) non-irradiation (b) local irradiation (c) whole body irradiation (d) whole body irradiation and injection of bone marrow cell group. H-E, 100×.](image1)

![Fig. 4. Histopathological features of new bone formation in the periosteum out of the socket. Three days after tooth extraction. (a) non-irradiation (b) local irradiation (c) whole body irradiation (d) whole body irradiation and injection of bone marrow cell group. Azan, 50×.](image2)

![Fig. 5. Histopathological features of new bone formation in the socket and in the periosteum out of socket. Five days after tooth extraction. (a) non-irradiation (b) local irradiation (c) whole body irradiation (d) whole body irradiation and injection of bone marrow cell group. Azan 13×.](image3)
newly formed bone in the whole body-irradiated rats into which bone marrow cells were injected (Fig. 3d). New periosteal bone formation was observed on the buccal side of the alveolar bone in the non-irradiation group on day 3 after the extraction (Fig. 4a). A large amount of newly formed bone with osteoblasts aligned on the surface, were observed between the upper alveolar bone and the perios- toematum. Although the periosteal new bone formation in the irradiation group was slightly delayed in comparison to that in the non-irradiation groups, the periosteal surface on the buccal side of the alveolar bone was histologically similar to the other groups (Fig. 4b–4d).

Newly formed bone occupied about one third of the distance from the bottom to the top of the socket wall in non-irradiation group on day 5 (Fig. 5a). Although the wound healing was slightly disturbed in the local irradiation group, it showed a tendency similar to that in the non-irradiation group (Fig. 5b). The extraction wound showed more delayed healing in the whole body irradiation group (Fig. 5c). The extraction socket was filled with granulation tissues, and there was little newly formed bone in the bottom of the socket. However, there was more newly formed bone in the whole body-radiated rats into which bone marrow cells were injected than that in the rats subject to whole-body irradiation.

![Fig. 6. Ultrasturactual features of osteoblast in the socket. Five days after tooth extraction. (a) non-irradiation (b) local irradiation (c) whole body irradiation (d) whole body irradiation and injection of bone marrow cell group. uranyl acetate and lead citrate stains. The bar shows 5 μm in each picture.](image)

![Fig. 7. The changes of number of ALP-positive osteoblasts within 500 μm × 500 μm square. (a) in bottom of the distal sockets. (b) on the periosteum out of the socket. The error bars show the standard deviations of the means.](image)

![Fig. 8. The number of ALP-positive osteoblasts within 500 μm × 500 μm square. (a) in bottom of the distal sockets, (b) on the periosteum out of the socket. The data (a) in bottom of the distal sockets show significant differences of numbers between only whole body irradiated rats and in the whole body irradiated rats injected with bone marrow cells. (P < 0.05).](image)
tion only (Fig. 5d). Transmission electron microscopy revealed that there were a number of activated osteoblasts on the bone surface with well-developed rough endoplasmic reticulum and Golgi apparatus in the non-irradiated rats (Fig. 6a). Osteoblasts in local irradiated rats were similar to those in the controls (Fig. 6b). Osteoblasts and osteocytes in the whole body irradiation rats were smaller and had vague cytoplasmic membranes (Fig. 6c). Irregular dilations of mitochondrial were shown and there was less rough endoplasmic reticulum. The cytoplasmic organelles had relatively increased in the osteoblasts in rats into which bone marrow cells were injected (Fig. 6d).

Whereas the number of osteoblasts increased day by day in the sockets of the non-irradiation and local irradiation groups, those in the whole body irradiation rats showed little change (Fig. 7a). Injection of bone marrow cells into the whole body-irradiated rats immediately after tooth extraction partially restored the number of osteoblasts (Fig. 8a). The number of osteoblasts on the newly formed periosteal bone increased rapidly in a similar way for 3 days after extraction and gradually decreased in association with the formation of new bone (Fig. 7b). There were no significant differences in the number of osteoblasts on the periosteum out of the socket between each experimental group on day 3 (Fig. 8b).

**DISCUSSION**

Granulation tissues containing fibroblasts and capillaries were found at the bottom of the extraction wound at day 2 after tooth extraction in the non-irradiation group, and new bone formation by osteoblasts was observed at day 3. The periosteum outside the extraction wound began thickening at day 1 after tooth extraction, and some new bone formation was observed at day 2. This was consistent with studies of normal extraction wound healing.11)

In addition, the wound healing process in the local irradiation group was similar to that in the non-irradiation group. On the other hand, the healing process after whole body irradiation was remarkably delayed even at the early phase, accompanied with poor granulation tissue formation and the appearance of fibroblasts and blood vessels. Eight Gy for whole body irradiation damaged bone marrow caused damage to bone marrow in the femurs of rats in a previous study.12) thus demonstrating a decreased number of marrow cells and many vacuoles caused by fatty deposits 7 days after the irradiation. Unlike the non-irradiation and local irradiation groups, delayed bone formation was observed inside the extraction wound of rats exposed to whole body irradiation. The decrease in the number of osteoblasts resulting from whole body irradiation recovered to some extent after intraperitoneal injection of femoral bone marrow cells. Therefore, the present results show that a certain number of pluripotent mesenchymal cells derived from bone marrow are essential for osteoblast differentiation and the subsequent trabecular bone formation.

Heterotopic bone stems from pluripotent mesenchymal cells, which have the ability to transform into osteogenetic stem cells.13,14) In addition, circulating osteoblast-lineage cells form bone in culture and in transplanted animals. Bone resorption by osteoclasts induced immature osteoblasts, while also disturbing the healing process in the socket. New bone formation outside the extraction wound occurs even if there is whole-body damage.11) New periosteal bone formation was not delayed much in the whole body irradiation group in the current study. This suggests that new periosteal bone formation is less associated with bone marrow cells, because it may not require coagulation and platelets. Periosteal cells may be at an advanced stage of cytodifferentiation and less sensitive to irradiation than pluripotent mesenchymal cells. The present results suggest that, if the origin of the cells is different, then the grade of complications would thus be different even if the cell type is the same. At present, it is difficult to explain these results only from viewpoint of a volume effect, because there was no difference in new periosteal bone formation between local irradiation and whole body irradiation. These findings showed that local irradiation is better than chemotherapy regarding the treatment of small solid cancer because whole
body complications cannot be avoided, as shown by the fact that chemotherapy would disturb wound healing.

REFERENCES


