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BIOLOGY CONTRIBUTION

EXPRESSION OF BONE MORPHOGENIC PROTEIN 2/4, TRANSFORMING GROWTH FACTOR-β_1, AND BONE MATRIX PROTEIN EXPRESSION IN HEALING AREA BETWEEN VASCULAR TIBIA GRAFTS AND IRRADIATED BONE—EXPERIMENTAL MODEL OF OSTEONECROSIS

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Purpose: For the surgical treatment of osteoradionecrosis after multimodal therapy of head-and-neck cancers, free vascular bone grafts are used to reconstruct osseous structures in the previously irradiated graft bed. Reduced, or even absent osseous healing in the transition area between the vascular graft and the irradiated graft bed represents a clinical problem. Inflammatory changes and fibrosis lead to delayed healing, triggered by bone morphogentic protein 2/4 (BMP2/4) and transforming growth factor (TGF)-β_1. Given the well-known fibrosis-inducing activity of TGF-β_1, an osteoinductive effect has been reported for BMP2/4. However, the influence of irradiation (RT) on this cytokine expression remains elusive. Therefore, the aim of the present *in vivo* study was to analyze the expression of BMP2/4, TGF-β_1, collagen I, and osteocalcin in the transition area between the bone graft and the graft bed after RT.

Methods and Materials: Twenty Wistar rats (male, weight 300–500 g) were used in this study. A free vascular tibia graft was removed in all rats and maintained pedicled in the groin region. Ten rats underwent RT with 5 × 10 Gy to the right tibia, the remainder served as controls. After 4 weeks, the previously removed tibia grafts were regrafted into the irradiated (Group 1) and nonirradiated (Group 2) graft beds. The interval between RT and grafting was 4 weeks. After a 4-week osseous healing period, the bone grafts were removed, and the transition area between the nonirradiated graft and the irradiated osseous graft bed was examined histomorphometrically (National Institutes of Health imaging program) and immunohistochemically (avidin-biotin-peroxidase complex) for the expression of BMP2/4, TGF-β_1, collagen I, and osteocalcin.

Results: Absent or incomplete osseous healing of the graft was found in 9 of 10 rats after RT with 50 Gy and in 1 of 10 of the rats with nonirradiated osseous grafts. Histomorphometrically, the proportion of osseous healing in the transition area was 17% in Group 1 and 48% in Group 2 ($p = 0.001$). Compared with the nonirradiated rats, reduced enchondral and perichondral ossification was found in the healing area after RT, with a reduction of BMP2/4 and osteocalcin expression. TGF-β_1 and collagen I expression in the transition area to the irradiated osseous graft bed was significantly increased compared with that in the nonirradiated osseous graft bed.

Conclusion: After RT, osseous healing of vascular bone grafts is significantly reduced and may be a result of radiation-induced inhibition of BMP2/4 and osteocalcin expression. In addition, induction of TGF-β_1 and collagen I expression occurs. Because the effects of the TGF-β superfamily are manifold and partially unknown, additional research directions could be in the exogenous application of BMP2/4 and inhibition of TGF-β_1 by antibody treatment to search for appropriate therapeutic approaches for improving osseous healing in the irradiated graft bed. © 2005 Elsevier Inc.

Osteoradionecrosis, Bone grafts, Irradiation, Bone healing, TGF-β_1, BMP, Osteocalcin, Collagen I.

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INTRODUCTION

Combined external beam radiotherapy (RT), with or without interstitial brachytherapy, and surgery are considered standard treatment approaches for head-and-neck cancer (1–4). Osteoradionecrosis of the mandible is not an uncommon late effect (5, 6). After hyperfractionated RT to a total dose of up to 83 Gy and conventional RT to a total dose of 60–70 Gy, osteoradionecrosis rates of 9–23% have been reported (5). The total dose, single fraction size, treatment technique, and interval between RT and surgery are of prognostic relevance for the development of osteoradionecrosis (5, 7–11). A significant increase in bone complications and, consequently, osteoradionecrosis was seen with a total mandibular dose of >69 Gy and particularly after combined external beam RT and interstitial brachytherapy (6).

One reason for a lower osteoradionecrosis rate after hyperfractionated RT to 83 Gy compared with normally fractionated RT to 70 Gy might be the induction of an apoptotic pathway of cellular elements at different periods.

In an animal model, irreversibly reduced bone healing ability was proven with a single dose of >15 Gy *in vivo* (11, 12). Preoperative, as well as postoperative RT up to the fourth postoperative day showed a significant reduction in bone healing (11) that resulted in an incomplete bone healing area, reduced bone torsion, and stiffness (13, 14). After radiation-induced apoptosis of osteoblasts, osteocytes, osteoprogenitor cells, and endothelial cells (11, 15), progressive hyalinization and fibrosis of the medullary spaces and reduction of osseous vascularization will occur. This in turn will lead to hypoxic, hypovascular, and hypocellular bone tissue. In the final stage, this process will eventually lead to ischemic bone necrosis (16–18).

For this reason, clinically preferentially vascular bone grafts are used for osseous reconstruction of previously irradiated bone during surgery (19–21). The grafting of vascular, osteoinductively effective bone tissue provides osteoblasts, osteocytes, and osteoprogenitor cells that induce bone remodeling and appositional bone regeneration. Transforming growth factor (TGF)-β_1 and bone morphogenetic proteins (BMPs) will induce the differentiation of mesenchymal progenitor cells to osteoprogenitor cells, which further differentiate to preosteoblasts and mature osteoblasts (22–25). Furthermore, the mitogenic and morphogenic effect of TGF-β_1 and BMP-2/4 on fibroblasts and osteoblasts induces the expression of components of the extracellular matrix (e.g., collagen and elastin fibers) and bone matrix proteins. In particular, collagen types I, II, IV, IX, and X are expressed in the proliferation phase as essential components of the bone matrix. Expressed osteogenic signals, such as osteocalcin, osteonectin, osteopontin, alkaline phosphatase, and bone sialoprotein induce the maturation of extracellular matrix components through mineralization of the bone matrix.

However, no data are available on the interrelationship of bone healing of vascular grafts in the previously irradiated graft bed and the endogenous expression of BMP-2/4, TGF-β_1, bone matrix proteins, and collagen I.

Therefore, using an animal model for osteonecrosis, we sought to answer the following questions: (1) to what extent does bone healing occur in the transition area between the irradiated bone and the vascular bone graft; (2) to what extent does an endogenous expression of BMP-2/-4 and TGF-β1 play a role; and (3) to what extent is the expression of osteocalcin and collagen I associated with bone healing in this model?

METHODS AND MATERIALS

Experimental design

Twenty male Wistar rats (weight 300–500 g, Charles River Wiga, Sulzfeld, Germany) were used in the study. The rats were kept in pairs in polycarbonate cages in accordance with the requirements of the German Animal Welfare Act (Animal Experiment Permit 621-2531.31-05/01) at a temperature of 22° ± 0.5°C at 55% humidity in 12-h light/dark cycles. They were given a standard pelleted rodent diet (No. 1320, Altromin, Lage, Germany) and water *ad libitum.*

The removal and transfer of the pedicled tibia graft to the groin, RT of the resulting bone defect, and the retransfer of the tibia graft into the previously irradiated removal defect were performed under inhalation anesthesia with isoflurane (Forene, Abbott, Wiesbaden, Germany) after modification of the circulation system of an anesthesia unit (Tiberius 19, Draeger, Bremen, Germany). For the 2 h of surgery, anesthesia was administered intraperitoneally with Ketanest (ketaminehydrochloride 0.1 mL/100 g KG, Pharmacia-Upjohn, Erlangen, Germany) and Rompur (xylazinhydrochloride 2%, 0.02 mL/100 g KG, Bayer, Leverkusen, Germany). Immediately before surgery, the rats were given intraperitoneal antibiotics consisting of 10,000 IU of a broad-spectrum penicillin (Tardomyocel, Bayer) and an intraperitoneal volume substitution of 15–20 mL electrolyte solution (Tuto-Op, Pharmacia-Upjohn).

Harvesting and transplantation of tibia graft

In all rats, a 1-cm-long tibia bone graft pedicled on the arteria and vena saphena was removed from the medial part of the right tibia with an oscillating saw (Martin, Nuremberg, Germany; Fig. 1). The donor site of the tibia was securely fixed with a titanium microplate with two screws each at the proximal and distal resection stump (Martin), bridged, and the pedicled tibia graft fixed subcutaneously in the groin outside a tattooed, later-irradiated volume. Perfusion was ensured macroscopically through bleeding from the osteotomy areas and the muscular cuff. Four weeks after the end of RT, the tibia graft, which had not been irradiated, was retransferred in a second operation from the groin into the previously irradiated removal defect and fixed to the bridge plate osteosynthetically. The rats were killed 4 weeks later, and the graft, including the osseous proximal and distal transition area, was removed.

Twenty rats with tibia grafts were evaluated in this study: 10 rats underwent RT to a total dose of 50 Gy (Group 1) and 10 rats did not undergo RT (Group 2). Macroscopically, plate loosening, wound healing disorders, inflammation, and macroscopically visible fibrous/osseous healing of the graft in the transition area to the preexisting resection edge were evaluated.