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Effects of irradiation on bone remodelling around mandibular implants: an experimental study in dogs

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Abstract. This research focuses on the effects of radiotherapy on bone remodelling around mandibular implants in dogs. After bilateral extraction of the mandibular premolars and first 2 molars, each of 11 beagles received 8 mandibular implants. Four animals were irradiated 4 weeks after implantation and 4 others 8 weeks before implantation; the remaining 3 did not receive radiotherapy. Irradiation consisted of 10 daily fractions of 4.3 Gy ^{60}Co. Fluorochromes were given at implantation and irradiation to allow the measurement of bone apposition. The dogs were killed 6 months after implantation. Each hemi-mandible was processed according to bone-specific histological techniques. New bone formation was visible around 85 of the 88 implants. Stimulated mandibular remodelling was attested in both irradiated groups by increased porosity and numerous labelled osteons. Resorption was more pronounced in the group irradiated after implantation, but osteon formation appeared unvarying. Osseointegration was thus shown to be compatible with bone irradiation as bone turnover activities were maintained throughout the experiment. As the apposition stage of the remodelling cycle appears crucial to achieve optimal osseointegration, its normal completion should be taken into account in clinical practice by respecting a 6-month period between irradiation and implantation.

Keywords: bone remodelling; dental implants; osseointegration; dogs; irradiation.

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Treatment of oral and maxillofacial cancers commonly consists of large surgical resections that necessitate bone and soft-tissue reconstruction. Oral implants are also used to restore esthetics and masticatory function. As the tumoural lesion can require radiotherapy, the skeletal tissues are confronted with both implant integration and radiation damage that is considered to result from hypocellularity, hypovascularity and hypoxia[20]. Many clinical studies report the placement of oral implants in irradiated bone, with a success rate varying from 75% to 99% after at least 5 years[6,12,21,27]. These results depend mainly on irradiation timing and dose, as well as on the site of implants[12,27]. The few experimental studies of bone reaction around oral implants in irradiated rabbits[18,24] and dogs[4,7] already carried out obtained relatively high levels of osseointegration,

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although less extensive than in non-irradiated animals. Experimental data on the rabbit suggested long-term impairment of bone remodelling after post-implantation irradiation[18,24]. In contrast, histological analysis of mandibular bone of dogs 24 weeks after implantation showed a higher level of remodelling in the irradiated animals than in the non-irradiated ones[8]. This qualitative observation was mostly restricted to the bone–implant interface. The present study, using the same experimental material as this latter paper, was designed to assess further the dynamics of cortical bone remodelling close to as well as at a distance from mandibular implants in irradiated dogs. In particular, the bone apposition efficiency in the osteons was measured using *in vivo* fluorescent markers of osteogenesis.

Materials and methods

Materials

Eleven male 1-year-old beagle dogs, of similar weight (about 12 kg) and size, were randomly assigned to 3 groups: a group of 4 dogs that were irradiated after implantation (IrA), a group of 4 dogs that were irradiated before implantation (IrB) and a control group of 3 non-irradiated dogs (C). Two different implants were selected for the present investigation: the Steri-Oss® (USA) submerged, hydroxyapatite-coated implant, diameter 3.8 mm and length 8 mm and the Straumann® (Switzerland) non-submerged implant with Ti plasma-sprayed coating, diameter 4 mm and length 8 mm.

Experimental procedure

In each dog, both mandibular premolars and the first 2 mandibular molars were extracted from each side so as to create 2 edentulous areas. Thereafter, 4 implants were placed in each edentulous area, alternating Steri-Oss® and Straumann® implants. In total, 88 implants were under study. Surgery was performed under Nembutal® (Abbott Laboratories, Belgium) general anaesthesia with laryngeal intubation and in sterile conditions. Intravenous cefazolin was used for antibiotic prophylaxis.

Two different experimental sequences were followed (Fig. 1). Groups C and IrA were implanted 8 weeks after extraction. In group IrA, the dogs were irradiated 4 weeks after implantation. The animals of group IrB underwent irradiation 6 weeks after extraction, then implantation 8 weeks after the end of radiotherapy.

Irradiation was delivered with a telecobalt therapy unit. A preliminary simulation was performed in order to allow reproduction of positioning across sessions by drawing precise landmarks on the dog's outer cheek. During the irradiation period, the animals were given daily intramuscular injections of ketamine hydrochloride for sedation. A daily dose of 4.3 Gy was administered for 10 consecutive days. The total dose of 43 Gy can be considered as equivalent to a total dose of 60 Gy delivered over a 6-week period in human radiotherapy, with 5 sessions a week[2].

As mucositis appeared in all dogs 1 week after the end of irradiation, 0.2% chlorhexidine digluconate mouth rinses were administered daily for 7 days. Two fluorescent intravital markers of osteogenesis were given (Fig. 1): intraperitoneal calcein green (30 mg/kg, Merck, Germany) at the time of implantation, and intramuscular terramycin (50 mg/kg) on the second day of irradiation or at the equivalent time in the non-irradiated dogs (group C).

The dogs were killed with a lethal dose of Nembutal® (Abbott Laboratories) after 24 weeks of implantation. The experimental protocol was approved by the local University Animal Care Committee. The guidelines for the care and use of laboratory animals were always observed.

Histological techniques

Each mandible was dissected out and divided into segments to isolate the implants and the surrounding bone. Each segment was fixed in 10% phosphate-buffered neutral formalin for 4 weeks, dehydrated in methanol, stained *en bloc* with 1% basic fuchsin[9] and embedded in methyl methacrylate without preliminary decalcification. After polymerization, each sample was cut with a diamond saw (Leitz, Germany) into serial sections, parallel to the main axis of the implant. The sections were polished and reduced to a uniform thickness of 80 μm with a rotating grinding machine (Planapol 2, Struers, Denmark).

Microradiographs were obtained by placing the sections in contact with a fine-grain emulsion (VRP-M, Slavich-Geola, Lithuania) exposed to long wavelength X radiations produced by a Machlett tube (Baltograph BF-50/20, Balteau, Belgium) at 14 kV and 15 mA. The exposure lasted 1 h for a film-focus distance of 106 mm. After development, the microradiographs were observed with an ordinary light microscope.

The sections were mounted with glycerin and examined under UV light microscopy. The osteons, which are the basic structural units of cortical bone[17,22], were recognizable by their concentric lamellar arrangement around a central Haversian canal. A green ring in osteon corresponded to calcein incorporation at the time of implantation, and a yellow ring to terramycin labelling at the beginning of the irradiation period (Figs 1 and 2, green and yellow arrows). Basic fuchsin stained the osteocytes (arrowheads) as well as the Haversian canal content (Fig. 2, red arrows).

Around each implant, 10 osteons including both fluorochromes were measured with a semi-automatic image analyser (MOP, Kontron, Germany). In each of these osteons, 3 surfaces were considered (Fig. 2). Surface 1 was delineated by the first fluorochrome, i.e. calcein green in groups IrA and C and terramycin in group IrB (Fig. 1). Surface 2 was outlined by the second fluorescent marker, i.e. terramycin in groups IrA and C and calcein green in group IrB. Surface 3 was the area of the Haversian canal, stained by fuchsin. The corresponding diameters (D1, D2, D3) were obtained as follows: diameter $= 2\sqrt{\text{surface}/\pi}$.

Statistical analysis was performed with SAS software (6.12, SAS Institute, USA). Repeated measures were averaged on a per-subject level. The authors then computed differences between means of groups defined by the experimental factor (irradiated versus non-irradiated, irradiated before or after implantation) with *t* test for independent samples (degrees of freedom proportional to number of dogs/group).

Fig. 1. Experimental timing (weeks) according to group. Extraction, implantation and injection of calcein, irradiation and injection of terramycin, death.