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Assessment of local blood flow with laser Doppler flowmetry in irradiated mandibular and frontal bone, an experiment in Göttingen minipigs

Lucas J. Poort [a, b, *], Monique M.F. Bloebaum [a], Roland A. Böckmann [a, b], Ruud Houben [c], Marlies E.P.G. Granzier [c], Frank J. Hoebers [b, c], Peter A.W.H. Kessler [a, b]

[a] *Department of Cranio-Maxillofacial Surgery, Maastricht University Medical Centre, Maastricht, The Netherlands*
[b] *GROW School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands*
[c] *Department of Radiation Oncology (MAASTRO), Maastricht University Medical Centre, Maastricht, The Netherlands*

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ABSTRACT

Purpose: The aim of this study was to investigate local blood flow changes in the mandibular bone compared to the os frontale after irradiation in various doses.

Materials and methods: This study used an animal experiment with 16 female Göttingen minipigs. Three groups of four animals were irradiated with equivalent doses of 25, 50 or 70 Gray on the mandible and os frontale and four animals served as control. Three months after irradiation laser Doppler flowmetry (LDF) was used to record local blood flow on the left mandible and in the irradiated area on the os frontale. At 6 months measurements were repeated. Descriptive and univariate analyses were conducted and p-values lower than 0.05 were considered statistically significant.

Results: Local blood flow measurements in the mandible were significantly higher compared to the os frontale. In the os frontale and mandible there was no significant change in the measurements with increasing irradiation dose.

Conclusion: We found a non-significant decrease in LDF values with an increase in radiation dose in the mandible and non-significant changes in the os frontale at 3 and 6 months. We consider this to represent the process of on-going fibrosis affecting the local blood flow in the mandible.

1. Introduction

In general, for head and neck malignancies, treatment regimens often include radiotherapy because of its beneficial effects on recurrence and survival (Klug et al., 2005; Paximadis et al., 2012; Ramaekers et al., 2011). However, irradiation effects on the surrounding healthy tissue continue to cause complications of varying severity (Lee and Moon, 2011; Thariat et al., 2011; Boomsma et al., 2012; Mul et al., 2012). One complication is osteoradionecrosis (ORN). The diagnosis is generally based on clinical presentation. ORN is defined as "irradiated bone, which becomes devitalized, is exposed through the overlying skin or mucosa, and does not heal within a period of 3 months without tumor recurrence" (Lyons and Ghazali, 2008). It can arise at any point after irradiation therapy (Marx and Johnson, 1987). ORN can affect all bony structures in the head and neck region but is most commonly seen in the mandible, at a frequency of 2%–22% (Teng and Futran, 2005).

Pathophysiology of ORN is, according to Delanian et al., a state in which radiation-induced fibrosis accounts for the damage observed in normal tissues, including bone, after radiotherapy. The destruction of endothelial cells coupled with vascular thrombosis leads to the necrosis of microvessels, local ischaemia, and tissue loss. This leads to the key event in this type of damage – the activation and deregulation of fibroblastic activity (Lyons and Ghazali, 2008).

The treatment of ORN ranges from conservative management, with or without surgical debridement, to broad resection in severe cases, a major surgical intervention (Jereczek-Fossa and Orecchia, 2002; Notani et al., 2003; Jacobson et al., 2010).

* Corresponding author. Department of Cranio-Maxillofacial Surgery, Maastricht University Medical Centre/Maastricht University MUMC+, Postbus 5800, 6202 AZ Maastricht, The Netherlands. Tel.: +31 43 387 2010; fax: +31 43 387 2020.
E-mail address: l.poort@mumc.nl (L.J. Poort).

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In earlier experiments, laser Doppler flowmetry (LDF) was used for recording microvascular blood flow in cancellous mandibular bone of young pigs (Hellem et al., 1983). LDF has also been shown to be useful in the assessment of bone vitality in osteomyelitis and in other applications (Swiontkowski et al., 1989; Duwelius and Schmidt, 1992; Teh, 2006).

LDF technique uses a laser diode with an operating wavelength of 780–820 nm, which is emitted into human tissues. Photons are scattered, and light hitting moving blood cells undergoes a shift in wavelength (Doppler shift) while the wavelength of light hitting static structures remains unchanged. A returning fibre in the probe collects the scattered light. The magnitude of the signal and the frequency changes is directly related to the relative number and velocity of blood cells in a given volume (Michelson et al., 1996).

To understand the effects of irradiation on blood flow in bone, we used an animal experiment already partially described in a previous publication (Poort et al., 2014). The irradiation and measurements were conducted on the os frontale and mandible. The os frontale and mandible have distinct anatomies and embryological origins (Carlson, 1999). The os frontale is composed almost entirely of dense cortical bone. Its vasculature is like a web with many anastomoses. The mandible has a dense outer cortex with loose trabecular bone in the marrow space. The vascularization of the mandible relies mainly on the inferior alveolar artery and lacks sufficient collaterals. This makes the mandible more vulnerable to vascular damage after irradiation (Lyons and Ghazali, 2008; Zhuang et al., 2011).

The aim of this study was to investigate changes in local blood flow of mandibular bone compared to os frontale after irradiation in various doses.

2. Materials and methods

The experiment was performed in accordance with the European Community Guidelines for the Protection of (laboratory) Animals as described in our earlier publication (Poort et al., 2014). Permission was obtained from the local Animal Ethical Committee (DEC 2011-127).

2.1. Study design

The study design was an animal experiment using 16 healthy, adult 18-month-old female Göttingen minipigs.

2.2. Study variables

Four animals were randomly assigned to each of the 4 research groups receiving an equivalent radiation dose of 25, 50 or 70 Gray (Gy) and one control group. The primary outcome variable was median LDF value. The primary predictor variables were radiation dose in different groups and measurement site. Other variables such as age and sex were constant for all animals.

2.3. Data collection methods

In preparing for the irradiation, for the three irradiated groups and the control group a planning CT-scan was made under general anaesthesia. A thermoplastic immobilization mask was employed for optimal fixation of the animal's head. A circular light metal wire was placed to mark the irradiation field on the os frontale. A Siemens Sensation Open™ CT-scan was used for radiological imaging (Siemens, Erlangen, Germany).

The animals were irradiated under general anaesthesia. The animals were positioned in individualized thermoplastics moulds for reproducible and steady positioning.

The irradiation was delivered using a 6 megavolt linear accelerator with two opposing lateral beams (Siemens, Erlangen, Germany). The entire body of the mandible was irradiated in two fractions with equivalent doses of 25, 50 or 70 Gy (two fractions of 6.5, 9.7, and 11.8 Gy, respectively). The same treatment was administered to the central portion of the os frontale with an external electron beam. An alpha/beta ratio for late responding tissues was used to calculate the equivalent dose. We assumed the alpha/beta ratio to be 3. The second fraction was given 48 h after the first fraction. In each group, every animal received the same equivalent irradiation dose for the mandible and for the frontal bone. None of the above was done in the control (0 Gy) group.

Thirteen weeks after irradiation, surgery was performed under general anaesthesia. The surgical procedure on the mandible involved extraction of all premolars and molars on the left side. Using a pilot drill of the implant system (BioComp Industries BV, Vught, The Netherlands), 4 initial holes were drilled in the residual alveolar ridge. To assess bone vascularity, laser Doppler flowmetry (Periflux System Perimed, Järfälla, Sweden) recordings were carried out at a depth of approximately 6 mm. After signal stabilization, a 20 s recording was carried out in every initial hole. Four recordings were made with the probe directed perpendicularly to the mesial, buccal, lingual and distal wall successively (Figs. 1 and 2). Four submerged dental implants (diameter 4.0 mm and length 12 mm) were placed in each pig. The measurements on the mandible took place on the side of the surgery in order to study both the effects of irradiation alone (in os frontale) and in combination with surgical intervention (in the mandible).

A small dose of methylene blue stain was injected into the periosteum of the frontal bone, marking the irradiated area. Then, an incision was made over the os frontale exposing the underlying bone. Four holes were drilled in the irradiated bone using the pilot drill and drill guide. To assess bone vascularity, LDF recordings were made at a depth of approximately 3 mm in the irradiated area (Figs. 3 and 4). The recordings were also conducted with the probe perpendicular to the wall of the initial hole in the above mentioned 4 directions. A median of all four values for each hole was later used for the statistical analysis. In that same area, 4 submerged dental implants (diameter 4.0 mm and length 4.0 mm) were placed in each pig. After rinsing the wound, it was closed in layers using resorbable sutures. The animals received

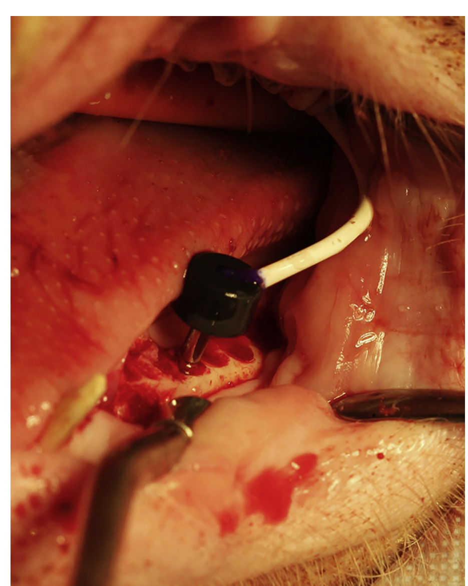

Fig. 1. Laser Doppler flowmetry measurements mandible.