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Hyperbaric oxygen therapy as a prevention modality for radiation damage in the mandibles of mice

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ABSTRACT

Background: Radiation therapy (RT) as part head and neck cancer treatment often leads to irradiation of surrounding normal tissue, such as mandibular bone. A reduced reparative capacity of the bone can lead to osteoradionecrosis (ORN). Hyperbaric oxygen therapy (HBOT) is used to treat ORN, based on its potential to raise the oxygen tension in tissues. However, prevention of radiation-induced damage is of great interest. Our purpose was to investigate whether HBOT could prevent radiation-induced damage to murine mandibles.

Methods: Twenty-eight mice were irradiated in the head and neck region with a single dose (15 Gy) and half of them were subsequently subjected to HBOT. Another 14 mice did not receive any treatment and served as controls. Ten and 24 weeks after RT, mandibles were harvested and analysed histologically and by microcomputed tomography (micro-CT).

Results: Micro-CT analysis showed a reduction in relative bone volume by RT, which was partly recovered by HBOT. Trabecular thickness and separation were also positively influenced by HBOT. Morphologically, HBOT suppressed the osteoclast number, indicating decreased resorption, and decreased the amount of lacunae devoid of osteocytes, indicating increased bone viability.

Conclusions: HBOT was able to partly reduce radiation-induced effects on microarchitectural parameters, resorption, and bone viability in mouse mandibles. HBOT could therefore potentially play a role in the prevention of radiation-induced damage to human mandibular bone.

1. Introduction

Radiation therapy (RT) is a standard component in the protocol treatment of head and neck cancer. Inevitably, normal surrounding tissues, including maxillofacial bones such as the mandible, will also be exposed to RT. Radiation damages small arteries, reducing the circulation to the already relatively poor vascularized mandible. This causes an impaired remodelling capacity of the bone and can lead to a reduction in bone mass and bone density, and thus to more vulnerable bone (Pacheco and Stock, 2013). The impaired reparative capacity of the bone especially poses a risk when trauma, such as subsequent surgery, biopsy, or tooth extraction and consecutive implant placement, is inflicted on the previously irradiated bone. Due to the vascular damage, a hypoxic and hypovascular environment exists in which bone is more prone to inflammation, which can eventually lead to destruction of bone, so called osteoradionecrosis (ORN) (Marx, 1983). ORN of the mandible is a serious long-term side effect in patients who receive radiation as part of the treatment for head and neck cancer. It is a very painful condition that can present itself even years after RT and is difficult to treat. Treatment regimens depend on the grade of ORN; lower grade can be treated effectively by long-term oral antibiotic therapy, whereas for more severe cases, removal of the affected bone might be necessary. Hyperbaric oxygen therapy (HBOT) is used as an adjuvant therapy to treat ORN, or is used in a preventive manner when minor or major surgery is performed in irradiated bone. The rationale is that HBOT, in which patients breathe 100% oxygen under elevated pressure, raises the oxygen tension and can thereby positively affect the healing process. Although clinical studies report positive effects, a general consensus about the effectiveness

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of the therapy remains to be achieved (Spiegelberg et al., 2010; Lubek et al., 2013). Experimental studies on the effects of HBOT on the treatment or prevention of ORN are especially scarce, although these kinds of studies would provide a better understanding of the effects and working mechanism of HBOT. Furthermore, the potential of HBOT to protect bone from radiation damage before complications have arisen has not been investigated thoroughly.

In this study, we investigated the effects of HBOT, when given directly after RT, on irradiated mandibular bone of mice, by means of micro–computed tomography (micro-CT) and histology. We were particularly interested in the effect on bone microarchitecture and viability in nontraumatised bone, to assess whether HBOT is able to prevent radiation-induced bone damage.

2. Methods

2.1. Animals

Female C3H mice (Harlan Netherlands BV, Horst, the Netherlands), 7–9 weeks old at the start of experimentation, were kept under standard housing conditions with free access to food pellets and acidified water. The mice were divided into three groups: control, radiation therapy (RT), and radiation therapy followed by hyperbaric oxygen therapy (RT + HBOT). At 10 and 24 weeks after RT, 7 mice of each group were sacrificed, and mandibles were harvested for ex vivo micro-CT scanning, after which they were used for histology. Animals were weighed frequently and given soft crushed food pellets to allow sufficient food intake after RT. The experimental protocol was approved by the Animal Care Committee of the Erasmus MC, Rotterdam, the Netherlands, under the National Experiments on Animals Act and adhered to the rules laid down in this national law that serves the implementation of "Guidelines on the Protection of Experimental Animals" by the Council of Europe (1986), Directive 86/609/EC.

2.2. Radiation- and hyperbaric oxygen therapy

Radiation therapy and hyperbaric oxygen therapy were given as previously described (Spiegelberg et al., 2014). In short, RT consisted of a single 15-Gy dose administered to the head and neck region of anesthetized mice.

Mice in the RT + HBOT group received the first HBOT session the day after RT. In an HBOT session, mice breathed 100% oxygen at 2.4 atm absolute during 1 h in a hyperbaric chamber suitable for small laboratory animals (Djasim et al., 2012). Twenty consecutive sessions were carried out daily, except at Saturdays and Sundays.

2.3. Micro-CT scanning

Immediately after mice were sacrificed by CO_2 asphyxiation, at 10 and 24 weeks after RT, mandibles were harvested and fixated in 10% buffered formalin. micro-CT was used to analyse bone parameters. micro-CT scans of the tissue blocks were made with a SkyScan 1076 in vivo microCT scanner (SkyScan, Aartselaar, Belgium) and the manufacturer's scanning software. Examination consisted of a scout view, selection of region of interest, off-line reconstruction, and evaluation. Serial transverse scan images were made at a resolution of 18 μm. Nrecon 1.3 (SkyScan, Aartselaar, Belgium) and CT Analyser 1.3.2.2 (SkyScan, Aartselaar, Belgium) software were used to reconstruct the data for analysis. A 3-dimensional volume of interest was created by applying interpolation between 2-dimensional free-hand selections of the mandibular bones. Within the volume of interest, the relative bone volume (BV/TV) was determined to quantify new bone formation, as well as trabecular number (TbN), trabecular thickness (TbTh), and trabecular separation (TbSp).

2.4. Histology

After micro-CT scanning, mandibles were decalcified in 10% ethylenediaminetetraacetic acid (EDTA) for 12 days, after which they were dehydrated and embedded in paraffin blocks. Slides (5 μm) were sagitally cut and standard haematoxylin eosin (HE) staining was performed. Per slide, the amount of empty lacunae and osteocytes were counted in 2–3 fields (×20 magnification). Empty lacunae were expressed as a percentage of the total count of osteocytes. Adipocyte density in bone marrow (number and area of adipocytes per square millimeter (mm^2) of bone marrow) was quantified using ImageJ version 1.45b (National Institutes of Health, Bethesda, MD, USA). Tartrate-resistant acid phosphatase (TRAP) staining was used to stain osteoclasts. Slides were first incubated for 20 min in 0.2 M acetate buffer with 50 mM L(+) tartaric acid (ICN Biomedicals Inc, Aurora, IL, USA). Then, 0.5 mg/ml naphthol AS-MX phosphate (Sigma–Aldrich Corp., St. Louis, MO, USA) and 1.1 mg/ml fast red TR salt (Sigma–Aldrich Corp., St. Louis, MO, USA) were added, after which slides were incubated for 60 min at 37 °C, rinsed in distilled water, counterstained with haematoxylin, and embedded with vectamount. The number of positively stained osteoclasts per millimeter of bone marrow perimeter was counted.

2.5. Statistical analysis

All data are expressed as mean values with standard error of the mean (SEM), and were analysed using SPSS PASW 21.0 for Windows (SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to test for normality, followed by the Mann–Whitney *U* test for the comparison of non–normally distributed data, whereas the Student *t* test was used for normally distributed data. Values of $p < 0.05$ indicated significant differences.

3. Results

3.1. Micro-CT

Fig. 1 shows bone parameters quantified by micro-CT scanning in the mandibles of mice, 10 and 24 weeks after radiation therapy. Fig. 1A shows the volume of interest, between the red lines, that was selected. At 10 weeks post-RT, no differences between groups were found in bone volume (Fig. 1B), trabecular thickness (Fig. 1C), trabecular separation (Fig. 1D), and trabecular number (Fig. 1E). However, porosity (Fig. 1F) was significantly increased in the RT group (36.6% ± 1.1%) compared to controls (31.3% ± 2.0%; $p < 0.05$). HBOT given after RT reduced the percentage of porosity towards control levels (30.7% ± 2.0%; $p < 0.05$). Twenty-four weeks after RT, no differences in the percentage of porosity were present anymore. However, the effect of RT became evident in the other parameters measured. Relative bone volume (BV/TV) significantly decreased in the RT-group (57.2% ± 0.6%) compared to control (63.0% ± 1.1%; $p < 0.01$). When HBOT was given after RT, relative bone volume increased towards control levels (62.2 ± 3.0; $p < 0.05$ for RT vs. RT + HBOT). The same pattern was seen for the trabecular thickness (control 0.171 ± 0.005 mm; RT 0.145 ± 0.003 mm; RT + HBOT 0.157 ± 0.007 mm; $p < 0.001$ for control vs. RT; $p < 0.05$ for RT vs. RT + HBOT). Trabecular separation was inversely affected, with a higher separation of trabeculae in RT (0.108 ± 0.002) compared to control (0.100 ± 0.002 mm; $p < 0.05$) and oppositely a reduction in trabecular separation due to HBOT (0.096 ± 0.008 mm; $p < 0.05$). Trabecular number was increased by RT (3.96 ± 0.06 vs. 3.70 ± 0.04