Journal of Cranio-Maxillo-Facial Surgery 43 (2015) 1829–1836

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

Development of mandibular osteoradionecrosis in rats: Importance of dental extraction

Pauline Bléry [a, b, c, d, e, *], Florent Espitalier [a, d, f], Alexandra Hays [a], Eléonore Crauste [a], Christelle Demarquay [g], Paul Pilet [a, d], Sophie Sourice [a], Jérôme Guicheux [a, d], Olivier Malard [a, d, f], Marc Benderitter [g], Pierre Weiss [a, b, d, 1], Noëlle Mathieu [g, 1]

[a] Inserm U791 (Head: Prof. P. Weiss), LIOAD, Faculté de Chirurgie Dentaire, 1 Place Alexis Ricordeau, 44042 Nantes Cedex 1, France
[b] Faculté de Chirurgie Dentaire (Head: Prof. Y. Amouriq), Université de Nantes, 1 Place Alexis Ricordeau, 44042 Nantes Cedex 1, France
[c] IRCCyN, CNRS 6597, IVC (Head: Prof. Patrick Le Callet), Polytech'Nantes, rue Christian Pauc, 44306 Nantes Cedex 3, France
[d] CHU Nantes, Pôle Hospitalo-Universitaire 4 OTONN (Head: Dr. G. Amador del Valle), 1 Place Alexis Ricordeau, 44093 Nantes Cedex 1, France
[e] Service d'Odontologie Restauratrice et Chirurgicale (Head: Prof. Y. Amouriq), CHU de Nantes, PHU4OTONN, 1 Place Alexis Ricordeau, 44093 Nantes Cedex 1, France
[f] Service d'Oto-Rhino-Laryngologie et de Chirurgie Cervico-Faciale (Head: Prof. P. Bordure), CHU de Nantes, Pôle Hospitalo-Universiatire 4 OTONN, France
[g] IRSN Institut de Radioprotection et de Sûreté Nucléaire, IRSN/PRP-HOM/SRBE/LR2I (Head: Dr. M. Benderitter), 31 avenue de la division Leclerc BP17, 92260 Fontenay aux Roses, France

ARTICLE INFO

Article history:
Paper received 10 June 2015
Accepted 17 August 2015
Available online 8 September 2015

Keywords:
Animal model
Irradiation
Mandibular
Micro-CT
Osteoradionecrosis
Tooth extraction

ABSTRACT

Objectives: To develop an animal model of mandibular osteoradionecrosis (ORN) using a high-energy radiation source (as used in human therapeutics) and to assess the role of tooth extraction on ORN development.
Materials and methods (study design): Ten animals were irradiated with a single 35- or 50-Gy dose. Three weeks later, the second left mandibular molar was extracted from three animals in each group. Nine weeks after irradiation, the animals were euthanized, with an injection of contrast agent in the blood-stream to highlight vascularization. Mandibles were harvested and studied using micro-CT, histology, tartrate-resistant acid phosphatase activity and scanning electron microscopy.
Results: This study demonstrates that a single 50-Gy dose associated with molar extraction is necessary for ORN development. In these conditions, absence of healing of the mucosa and bone, dental effects, fibrosis, an increase in osteoclast activity and a decrease in vascularization were observed. We also determined that molar extraction increases the impact of the cellular effects of radiation.
Conclusion: The mandibular ORN animal model was validated after 50-Gy irradiation and molar extraction. The results of this study therefore support an animal ORN model and tissue engineering strategies will now be developed to regenerate bone for patients with head and neck cancer.

1. Introduction

In France, carcinomas of the upper aerodigestive tract account for approximately 12% of cancers. The treatment is based on wide surgical resection associated with external radiation therapy and sometimes chemotherapy (Institut National du Cancer (INCA), 2011). The consequences of treatment can be severe, especially from an aesthetic and functional point of view (Jegoux et al., 2010; Epstein et al., 2012). Mandibular osteoradionecrosis (ORN), which occurs in approximately 5% of patients who undergo radiotherapy, is considered as an extensive and irreversible bone necrosis, secondary to ionizing radiation (Marx, 1983; Epstein et al., 1987; Balogh and Sutherland, 1989; Dambrain, 1993; Delanian and Lefaix, 2007; Lyons and Ghazali, 2008; Madrid et al., 2010; Lyons et al., 2014). Diagnosis of ORN is based on clinical signs of mucosal ulceration with exposure of necrotic bone, without healing for 3–6 months (Marx, 1983; Epstein et al., 1987; Chrcanovic et al.,

* Corresponding author. Inserm U791 (Head: Prof. P. Weiss), LIOAD, Faculté de Chirurgie Dentaire, 1 Place Alexis Ricordeau, 44042 Nantes Cedex 1, France.
E-mail addresses: pauline.blery@univ-nantes.fr, pauline.blery@free.fr (P. Bléry).
[1] Co-last authors.

http://dx.doi.org/10.1016/j.jcms.2015.08.016
1010-5182/

2010). Many theories exist on the pathophysiology of ORN. Marx first hypothesized the '3 Hs': hypoxia, hypovascularization, and hypocellularity, for the development of ORN. Moreover, ORN is defined as non-healing exposed bone associated with a variable incidence of pain, orocutaneous fistulae or pathological fractures. A minor role of micro-organisms and a direct role of trauma have also been advanced (Marx, 1983). Dambrain (1993) advanced the notion of '2 Is': ischemia and infection in the development of the pathology. Delanian and Lefaix (2004, 2007) have advanced the radiation-induced fibrosis theory of formation of free radicals, endothelial dysfunction, inflammation, microvascular thrombosis and fibrosis. Currently, mandibular ORN is diagnosed when many criteria are found: exposed bone for at least 3 months, radiation therapy in the region, and a zone of necrotic bone without cancer recurrence. Clinical data and results of animal experiments of mandibular ORN show cell damage (endothelial and bone cells), vascular destruction (thrombosis of vessels), and an increase in osteoclast activity associated with bone marrow fibrosis and adiposity (Marx, 1983; Bras et al., 1990; Dambrain, 1993; Dudziak et al., 2000; Jereczek-Fossa and Orecchia, 2002; Fajardo, 2005; Delanian and Lefaix, 2007; Cohen et al., 2011; Chandra et al., 2014). The same conclusions were made based on different experiments, but the mechanism is not perfectly understood, and many of these experiments were conducted with low-energy X-rays. This study used high-energy X-ray technology, used in human therapeutics, to recreate the same bone damage. The aim of the present study was therefore to develop an animal model of mandibular ORN in rats, to better understand the pathophysiological mechanisms and to develop longer-term tissue engineering strategies.

2. Materials and methods

2.1. Animals

Seventeen 8-week-old Sprague–Dawley rats, provided by a certified breeding centre (Charles River, BP 0109, F 69592, L'Arbresle, France) were used for this study. The animals were received at the IRSN (Institute for Radiological Protection and Nuclear Safety) animal supplier (No. C92-approval 032-01 issued June 23, 2011). Protocols were submitted to the IRSN ethics committee and to the Pays de la Loire ethics committee with approval number CEEA.2012.82, in accordance with the European directive (DE 86/609/CEE; modified DE 2003/65/CE) for conducting animal experiments.

One week of acclimatization was respected. The animals were housed in ventilated cages with a double level (three animals per cage according to European standards). The animals were carefully monitored (behaviour and food intake) and the animals were weighed weekly throughout the experiment. At day 0, ten animals were irradiated and seven animals were used as controls. Three weeks after irradiation, the second left mandibular molar was extracted. Nine weeks after irradiation, the animals were euthanized with injection of contrast agent in the bloodstream to study vascularization. Mandibles were harvested for qualitative and quantitative analysis.

2.2. Radiation delivery procedure

The external radiation delivery was performed with Alphée, at the Institute for Radiological Protection and Nuclear Safety, Fontenay-aux-Roses, France, under general anaesthesia. Alphée is an accelerator-type radiation source (maximal energy is 4 MeV with an average energy of about 1.5 MeV; 30 kA). Isoflurane anaesthesia (Forène, Abbott France, Rungis, France) was delivered during irradiation, 5% for induction and 1% for preservation. The radiation doses delivered were 35 Gy ($n = 5$) or 50 Gy ($n = 5$) in a single dose (dose rate 2.4 Gy/minute). Animals were positioned on the left side in a window of radiation measuring 2 cm by 3 cm, allowing only irradiation of the mandible.

2.3. Dental extraction

The extractions were performed 3 weeks after irradiation under general anaesthesia (ketamine 100 mg/kg (Virbac) and xylazine 10 mg/kg (Rompun 2, Bayer, Leverkusen, Germany) by an intraperitoneal injection). The surgical procedure proceeded as follows: dermal and oral disinfection, mouth opening, detaching the gingiva around the second left mandibular molar and extraction of the tooth. Three irradiated animals per group underwent tooth extraction as did three control animals. An intramuscular injection of buprenorphine (10 μg/kg; Buprecar, Animal Care, York, UK) was immediately given as postoperative analgesia.

2.4. Injections of contrast agent and euthanasia

Under general anaesthesia with isoflurane, after disinfection, the abdominal cavity and the thorax were opened. A catheter was connected to the left ventricle of the heart; the second end of the catheter was connected to a warmed (40 °C) barium Sulfate mixture. The contrast agent was previously prepared using 50% barium Sulfate (Micropaque, Guerbet, Roissy CdG Cedex, France) and 1.5% gelatin (Sigma–Aldrich St. Louis, MO, USA) in phosphate buffered saline (PBS). After section of the right atrium, approximately 200 ml of contrast agent was injected into the bloodstream at a regular flow with a peristaltic pump (750 ml/h), until all the animal extremities became white. The animals died, under anaesthesia, a few minutes after the barium Sulfate injection was started. After injection, mandibles were harvested and fixed in a solution of paraformaldehyde.

2.5. Micro-CT scanning and image analysis

Mandibles were scanned using micro-CT at a resolution of 6 μm. The micro-CT used is a Skyscan 1272 (Skyscan, Bruker, Belgium). The acquisition parameters were: 100 kV, 100 mA, 0.5-mm aluminium filter, rotation step at 0.45° on 180°. The NRecon software (Skyscan) was used for reconstruction.

2.6. Histological examinations

The non-decalcified explanted bone specimens were fixed for 72 h in a 4% paraformaldehyde phosphate-buffered saline (PBS, Seroderm, Berlin, Germany), then dehydrated through a graded series of ethanol and acetone and embedded in Technovit resin (low temperature methyl methacrylate resin, Technovit 9100 NEW, Kulzer, Germany), used in the destabilized form. Different pre-infiltration and infiltration steps were carried out before embedding the samples in the polymerization mixture at −20 °C for 5 days until the end of the polymerization process. After being stored at 4 °C, the samples could be used at room temperature. Serial 5-μm sections were cut from two points of the mandibles in the frontal direction (forward molars and at the extraction zone) using a microtome made for non-decalcified tissues (Polycut Leica SM2500, Wetzlar, Germany). The bone sections were stained with Movat pentachrome and hematoxylin and eosin. The TRAP (tartrate-resistant acid phosphatase) activity was marked (controls were done on spleen sections). Osseous defects, the extraction zone, the number of blood vessels, osteoclast activity and teeth were observed under a light microscope (Axioplan 2, Zeiss, Oberkochen, Germany).