Journal of Cranio-Maxillo-Facial Surgery 43 (2015) 1823–1828

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

Perioperative discontinuation of intravenous bisphosphonate therapy reduces the incidence and severity of bisphosphonate-related osteonecrosis of the jaw: A randomized, controlled, prospective experimental study in rats

Mohammad Zandi [a, b, *], Arash Dehghan [c, 1], Khaled Ghadermazi [a, 2], Hamid Malekzadeh [a, 3], Mahdi Akbarzadeh [d, 4]

[a] Department of Oral and Maxillofacial Surgery, Hamedan University of Medical Sciences, Hamedan, Iran
[b] Dental Research Center, Hamedan University of Medical Sciences, Hamedan, Iran
[c] Department of Pathology, Hamedan University of Medical Sciences, Hamedan, Iran
[d] Department of Biostatistics and Epidemiology, Hamedan University of Medical Sciences, Hamedan, Iran

ARTICLE INFO

Article history:
Paper received 15 May 2015
Accepted 12 August 2015
Available online 20 August 2015

Keywords:
Bisphosphonate
Osteonecrosis
Jaw
Drug holiday
Animal study

ABSTRACT

Objective: To evaluate the effects of intravenous bisphosphonate discontinuation on incidence and severity of bisphosphonate-related osteonecrosis of the jaw (BRONJ).
Material and methods: Seventy rats were randomly divided into 7 groups. In control and S0 groups, weekly injection of saline and 0.06 mg/kg zoledronate (respectively) for 4 weeks, tooth extraction, continuation of injections for 2 months and euthanasia were performed. In group S1, zolendronate injection for 4 weeks, tooth extraction, zolendronate discontinuation for 2 months, and euthanasia were done. For groups S2, S3, S4, and S5, zolendronate injections for 4 weeks, drug holiday for 1–4 months (respectively) before and 2 months after tooth extraction, and euthanasia were performed. Presence of bone exposure, osteonecrosis, and new bone formation were clinically and histologically evaluated.
Results: The rate of BRONJ in control, S0, S1, S2, S3, S4, and S5 groups was 0%, 85%, 80%, 65%, 60%, 50%, and 40%, respectively. In control group, epithelial healing, bone formation, and absence of osteonecrosis; and in S0 group, unhealed epithelium, osteonecrosis, and impaired bone formation were histologically observed. In study groups, prolongation of drug holiday caused diminished osteonecrosis, and improved bone and epithelial healing.
Conclusion: Zolendronate discontinuation significantly decreased the incidence and severity of BRONJ in rats.

1. Introduction

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a well-documented serious complication associated with the use of nitrogen-containing bisphosphonates (BPs). BRONJ is defined as exposed bone or bone that can be probed through an intraoral or extraoral fistula in the maxillofacial region that persists for more than 8 weeks in patients who are currently receiving or who previously received BPs, with no history of head and neck radiotherapy or obvious metastatic disease to the jaws. Because osteonecrosis of jaws had been observed in patients receiving other antiresorptive and antiangiogenic medications, the term "medication-related osteonecrosis of the jaw" (MRONJ) has been recently introduced (Ruggiero et al., 2014).

In spite of considerable progress that has been made toward the elucidation of the etiopathology, associated risk factors, and management of BRONJ, the concrete preventive and therapeutic strategies for this devastating disease are still controversial. In previous

* Corresponding author. Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Hamedan University of Medical Sciences, Shahid Fahmideh Street, Hamedan, Iran. Tel.: +98 81 38234047, +98 9181111488 (mobile); fax: +98 81 38234014.
E-mail address: zandi88m@yahoo.com (M. Zandi).
[1] Tel.: +98 9122115786. a.dehghan50@yahoo.com
[2] Tel.: +98 9183141428. ghadermarzi593@gmail.com
[3] Tel.: +98 9173065089. hamid_malekzadeh_28@yahoo.com
[4] Tel.: +98 9187014593. akbarzadeh.ms@gmail.com

http://dx.doi.org/10.1016/j.jcms.2015.08.008
1010-5182/

studies, a variety of preventive measures for BRONJ have been recommended, including use of alternative dosing schedules that reduce BP exposure; avoidance of surgical interventions that might cause bone exposure, drug cessation before and after tooth extraction, perioperative antibiotic prophylaxis, and immediate mucoperiosteal coverage if tooth extraction is inevitable (Saia et al., 2010; Abtahi et al., 2013; Ruggiero et al., 2014; Bodem et al., 2015; Otto et al., 2015).

Tooth extraction in oncologic patients under treatment with intravenous BPs is reportedly associated with a high risk of BRONJ development (Voss et al., 2012; Pichardo and van Merkesteyn, 2013; Vercruysse et al., 2014), and it is not known whether discontinuation of BP before or after surgical intervention reduces the risk of BRONJ in this group of patients (Saia et al., 2010; Ruggiero et al., 2014). The rationale behind BP cessation is the hypothesis that BPs inhibit osteoclastic activity and decelerate bone turnover, and their discontinuation is expected to promote osteoclastic function recovery and improve bone turnover and healing (Ruggiero et al., 2014; de Molon et al., 2015). However, the current knowledge about the efficacy of a “drug holiday” on BRONJ prevention and treatment is based mostly on observational studies and on case reports and series.

Considering the prevalent use of intravenous BPs, the high risk of BRONJ associated with these medications, the difficult management of this complication, the role that a perioperative drug holiday may play in prevention of this serious disease, and the paucity of related evidence-based information in the literature, the authors of the present investigation were encouraged to perform this experimental animal study.

The aim of the present study is to evaluate whether perioperative stopping of the intravenous BP therapy reduces the incidence and severity of BRONJ in a rat model.

2. Material and methods

The protocol for this randomized, controlled, prospective experimental research was reviewed and approved by the Hamedan University of Medical Sciences Ethics Committee.

A total of 70 Wistar Albino rats weighing 300–350 g were obtained from the Animal House of the University and used in this experiment. The animals were acclimatized in the animal house for 10 days before the commencement of the experiment.

The rats were randomly divided into 7 groups: 6 study groups of S0 to S5 (10 in each) and a control group (10 rats). The rats in the S0 group received intraperitoneal injection of 0.06 mg/kg zoledronate (Zometa, Novartis Pharma, Basel, Switzerland), and the rats in the control group received the same volume of normal saline solution, once a week for 4 weeks (a total of 5 injections). Then, the rats underwent bilateral extraction of the mandibular first molar. The injections were continued for 2 months after tooth extraction, and then the animals were sacrificed. The rats in study group of S1 received the same dose of zolendronate as in the S0 group for 4 weeks, and then underwent tooth extraction. The drug was discontinued for 2 months after tooth extraction and the rats were sacrificed. Rats in the study groups of S2 to S5 received the same dose of zolendronate for 4 weeks; the drug was discontinued for 1, 2, 3 and 4 months before tooth extraction, respectively; the drug holiday was continued for 2 months after tooth extraction, and the rats were sacrificed. The outline of the study design is illustrated in Fig. 1.

Tooth extraction was conducted under intraperitoneal general anesthesia using 75 mg/kg of ketamine hydrochloride (Rotexmedica, Trittau, Germany) and 7.5 mg/kg of midazolam (Midazolex, Exir, Iran). With the use of a sharp dental explorer, the gingiva around first molars was detached and the teeth were extracted. Eight weeks after tooth extraction, using an overdose of anesthetic, all 70 rats were euthanized and clinically examined for the presence of bone exposure at the extraction sites and intraoral or extraoral fistulae that could be probed to bone (as defined for diagnosis of BRONJ). The mandibles were then extracted, sectioned at the midline, and the 140 hemimandibles fixed in 10% formalin solution, decalcified with ethylenediaminetetraacetic acid (EDTA), and embedded in paraffin. Histological sections (serial 4-μm slices) of the mandible at the tooth extraction area were cut parallel to the long axis of the adjacent teeth, and stained with haematoxylin and eosin. A pathologist who was blinded to the study design and coding system of the samples evaluated the sections for the presence of necrosis (more than 8 contiguous lacunae without osteocyte) in bone around extraction site and new bone formation in the extracted tooth socket.

Using SPSS 16.0 software (SPSS Inc., Chicago, IL), statistical analysis was done by Pearson's chi-squared and Fisher's exact tests, and $p < 0.05$ was interpreted as statistically significant.

3. Results

All 70 rats tolerated the experiment well. The frequencies of clinical bone exposure/fistula and histological osteonecrosis and bone formation in the studied groups are presented in Table 1.

In the control group, clinical examinations demonstrated no bone exposure or fistula. Histological examination of the extraction sites showed new trabecular bone formation in the extraction socket and absence of necrosis in the adjacent bone (Fig. 2A).

In the S0 group, bone exposure or fistula was observed in 85% of the rats. In some cases, osteonecrosis extended to the inferior border of the mandible and involved the skin. In histological examination of this group, epithelial discontinuity, inflammatory infiltrate, sequestra, impaired new bone formation in the extraction socket, and necrosis of the surrounding alveolar bone were frequently observed (Fig. 2B).

The frequency of clinical bone exposure/fistula in groups S1, S2, S3, S4, and S5 was 80%, 65%, 60%, 50%, and 40%, respectively. Histological examination showed that from group S1 toward group S5, the area of osteonecrosis was diminished, and granulation tissue formation and organization, fibroblast proliferation, new trabecular bone formation, and epithelial healing were more commonly observed (Fig. 2C).

In present study, as the duration of drug holiday was increased (from group S0 toward group S5), a decrease in the incidence rates of the bone exposure/fistula, osteonecrosis, and an increase in the rate of new bone formation were observed (Fig. 3).

Comparison of the rates of bone exposure/fistula, osteonecrosis, and new bone formation between study groups of S1 to S5 and the S0 group are presented in Tables 2–4. The rates of clinical bone exposure/fistula and osteonecrosis in groups S4 and S5, and new bone formation in groups S3 to S5 were significantly different from that in the S0 group ($P < 0.05$).

4. Discussion

To date, several strategies for prevention of BRONJ in BP users needing tooth extraction have been published in the literature, including the use of nonsurgical dental treatments such as root canal therapy to avoid tooth extraction, perioperative antibiotic prophylaxis, and the use of alternative dosing regimens that might decrease the risk of BRONJ (Marx et al., 2007; Dimopoulos et al., 2009; Saia et al., 2010; Abtahi et al., 2013; Ruggiero et al., 2014; Bodem et al., 2015; Otto et al., 2015); one of the proposed strategies is the perioperative discontinuation of the BP therapy. It is asserted that BPs inhibit osteoclastic activity and suppress bone