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The Potential Role of Angiogenic Osteoclast Inhibition in the Occurrence of Bisphosphonate-Related Osteonecrosis of the Jaw

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Summary

Bisphosphonate-related osteonecrosis of the jaw is a serious complication of systemic administration of bisphosphonate, the mechanism of which is still unclear.

Many hypotheses concerning pathophysiology are discussed, the most cited of which are: suppression of bone turnover and suppression of angiogenesis, but neither would explain all of the unique features of bisphosphonate-related osteonecrosis of the jaws.

Bisphosphonates are powerful osteoclast inhibitors, and recent studies have shown that osteoclasts are important for bone angiogenesis. Therefore, we hypothesize that bisphosphonates inhibit osteoclastic stimulation of angiogenesis, thus contributing to the occurrence of osteonecrosis of the jaws.

This theory would partially explain to the unfathomable, the pathophysiology of osteonecrosis of the jaw linked to bisphosphonates.

Introduction

Bisphosphonates are endogenous pyrophosphate analogues with a strong affinity for hydroxyapatite crystals. They inhibit osteoclast activity, disrupt osteoclast-mediated bone resorption, and reduce the lifespan of osteoclasts [1, 2]. They are used in the treatment of a wide variety of diseases, including osteoporosis, bone metastases and Paget's disease [1]. Bisphosphonate-related osteonecrosis of the jaw is a contemporary but well-codified clinical entity [3]. The American Association of Oral and Maxillofacial Surgeons (AAOMS) defines bisphosphonate-induced osteonecrosis of the jaw as the exteriorization of parts of the jaw in patients who have been exposed to bisphosphonates that has persisted for more than eight weeks without a history of jaw radiation therapy [4].

The main risk factors for the development of bisphosphonate-related osteonecrosis of the jaw are the potency of the bisphosphonates and the cumulative dose. Amino bisphosphonates are significantly more associated with osteonecrosis of the jaw than non-amino bisphosphonates, and intravenous administration increases the risk of developing osteonecrosis of the jaw up to 4.4-fold [5, 6]. Patients taking bisphosphonates monthly for oncologic purposes have a prevalence rate of 3-7% of developing osteonecrosis of the jaw [7, 8].

In addition, recent studies have suggested the involvement of other factors: dental extractions, microbial biofilms or infection of the

oral cavity [5, 9] however further evidence is needed to determine whether infection is primary or ancillary in the pathophysiology of bisphosphonate-related osteonecrosis of the jaw.

The pathogenesis of osteonecrosis of the jaw related to bisphosphonates is still poorly understood since it was brought to light in 2003 [10], despite the diversity of reports on the subject. Several postulates have been proposed, including bone remodelling and suppression of angiogenesis [11]. Nearly all reports mentioned the suppression of bone remodelling as the primary mechanism of action of bisphosphonates.

Nevertheless, the greatest challenge to the suppression-reform hypothesis can be summarized in three aspects: Firstly, bisphosphonate-related jaw osteonecrosis has not been reported in patients treated with other remodelling agents; secondly, bisphosphonate-related jaw osteonecrosis occurs only in the jaws and not in other long bones or vertebrae; thirdly, intravenous administration significantly increases the risk of developing this condition. The suppression of angiogenesis is another popular theory. Prior to the development of bisphosphonate-related osteonecrosis of the jaw, knowledge focused on avascular necrosis of the hip and osteoradionecrosis, both of which occurred as a result of a disruption of the vascular system. Suppression of angiogenesis plays an important role in the development of osteonecrosis, but is this as true in bisphosphonate-related jaw osteonecrosis? Numerous in vitro and a small number of in vivo studies have documented the direct antiangiogenic effects of bisphosphonates [12, 13].

Suppression of angiogenesis readily explains the higher morbidity of jaw osteonecrosis associated with intravenous bisphosphonates. In addition, like avascular necrosis of the hip and osteoradionecrosis, bone exposed in bisphosphonate-induced osteonecrosis of the jaw does not bleed on entry and is obviously avascular. However, more potent antiangiogenic drugs in clinical use today have not been shown to produce osteonecrosis, with the exception of two cases of exposed bone in the mandible, similar in nature to bisphosphonate-related osteonecrosis of the jaw, that have been reported in cancer patients treated with bevacizumab, a recombinant human monoclonal antibody that binds to vascular endothelial growth factor and inhibits angiogenesis [14]. The two cases alone imply that there should be some process other than the direct antiangiogenic effect of bisphosphonates. Furthermore, the direct antiangiogenic effects of bisphosphonates cannot consistently explain why bisphosphonate-related osteonecrosis occurs only in the jaws.

Hypothesis

Our hypothesis is that bisphosphonates may inhibit the stimulation of angiogenesis osteoclasts, which play an important role in the development of bisphosphonate-related osteonecrosis of the jaw. The article ''Osteoclasts are important for bone angiogenesis'', which was recently published in The Blood, reported that osteoclasts contribute to angiogenesis in vitro and in vivo by a mechanism requiring matrix metalloproteinase-9 (MMP-9) [15,16]. Prior to this study, the role of osteoclasts in angiogenesis was unclear, with no studies showing that osteoclasts stimulate angiogenesis in vivo. Both osteoclastogenesis and angiogenesis are enhanced in pathological conditions such as multiple myeloma, bone metastases and rheumatoid arthritis [17, 18]. Osteoclasts and blood vessels are closely associated with a vessel in each bone remodeling compartment [19]. Osteoclast-conditioned media have been reported to be angiogenic in vitro [20, 21]. Zoledronic acid, altered angiogenesis by targeting MMP-9 expressing macrophages [22].

The evidence suggests that osteoclast formation and/or activity and angiogenesis are linked in both bone development and remodeling, and provides strong evidence that suppression of angiogenic osteoclast activity may play a role in the development of bisphosphonate-related osteonecrosis of the jaw. Our hypothesis could help explain to the unintelligible why this condition has been rarely reported in patients treated with other antiangiogenic agents. The latter do not have significant inhibitory effects on osteoclasts, so direct antiangiogenic effects rarely occur [14]. Especially in trauma, suppression of osteoclast angiogenic activity may more easily lead to osteonecrosis.

Why does bisphosphonate-related osteonecrosis only occur in the jaws and not in other long bones or vertebrae? One plausible reason is that osteoclasts are less important than osteoblasts for the angiogenesis of vertebrae and long bones [15]. It has been reported that the contribution of osteoclasts and osteoblasts to angiogenesis differs at different anatomical sites, and that osteoblasts have

considerable function in long bones than craniofacial bones [23]. This means that even if the angiogenic activity of osteoclasts was inhibited, long bones or vertebrae could survive because of the contribution of osteoblasts to angiogenesis. Bisphosphonate-related osteonecrosis therefore occurs only in the jaws where osteoclasts dominate angiogenesis.

The other explanation could be the involvement of other local and systemic factors regulating angiogenesis, which remain to be clarified. In addition, our hypothesis explains the higher morbidity with intravenous administration, since it is in fact an adjunct to the hypothesis of suppression of angiogenesis.

To solve this problem, we established a protocol to test our hypothesis. The aim is to determine whether the combined use of nitrogen-containing osteoclast inhibitors and non-nitrogen-containing antiangiogenic agents could lead to a high incidence of osteonecrosis of the jaw. The results of this study will be published in a separate paper and may provide evidence of the pathophysiology of bisphosphonate-related osteonecrosis of the jaw.

Materials and Methods

It will be an experimental study, conducted over a period of 4 months in the animal house of the Faculty of Medicine and Biomedical Sciences in Yaoundé. The sample will be composed of 31 male and female rats (wistar strains) with an average age of weeks and an average weight of 250 g (range 210-382 g).

Animal Care and Osteonecrosis Induction

The animals will be placed in appropriate plastic cages, labelled and ventilated, with cycles at controlled temperature (22+/- 1°c) and light (from 7 to 19 hours). Bedding will be changed 3 times a week. Food and filtered water will be provided at will. Body weight will be measured once a week.

After 2 weeks of acclimatization, the animals will be randomly divided into three groups according to whether or not they are taking a bone antiresorptive:

- Group I: will receive clodronate + dexamethasone subcutaneously
- Group II: will receive intraperitoneally zoledronic acid + dexamethasone
- Control group: will receive a placebo (saline solution).

The experiments will be conducted between 9:00 am and 5:00 pm with minimal diurnal variation. Clodronate will be administered once a week at cumulative doses of 36, 84 and 300 mg/kg for 3 months (24). Group II animals will receive 5 doses of zoledronic acid 0.6 mg/kg intraperitoneally at 28-day intervals (25). The two groups described above will receive 1 mg / kg (0.25 g / 250 g) of dexamethasone intraperitoneally. A saline solution of equal volume with the other drugs will be administered to the control group for 6 weeks. The doses and timing of drug administration are designed in accordance with studies in the literature.

Clinical Assessment

To determine the presence/absence of oral lesions, a blind observer in the study will conduct a thorough oral examination.

Dental Extractions

All surgeries will be performed blind by a single operator. The time of each surgery has been recorded.

All surgeries will be performed blind by a single operator. The time of each surgery has been recorded. Dental extractions will be performed 45 days after the start of the experiment, under general anaesthesia with a mixture of ketamine (100 mg/kg) and Diazepam (8 mg/kg), Atropine (0.4 mg/kg) administered peritoneally (8). Extraction of the first mandibular molar will be performed in the same manner as in the control group. The animal will be placed in the supine position, the mouth will be held open by means of elastics (type of ropes) anchored to the upper and lower incisors, and stretched and fixed to the back. The operating table.

0.1 ml of 2% lidocaine hydrochloride with epinephrine 1: 200 000 will be injected into the oral and lingual mandibular mucosa. 0.075 mg/kg will be administered daily subcutaneously for three postoperative days [25]. During the surgical procedure, the area will be permanently irrigated with saline and closed with resorbable sutures. Nine weeks after the extractions, two animals from each group will be euthanized under general anaesthesia (isofluorane inhaled with oxygen at 3 L/min) [8]. The heads will be harvested as a block by decapitation for macroscopic and microscopic analysis. The animals will be weighed daily throughout the study.

Histological Analysis

The jaw of each euthanized rat will be removed: posterior mandible, anterior mandible and femur (as a control site). The pieces will be placed in a 10% buffered formalin solution. After fixation, the bone samples will be decalcified in 25% formic acid. The samples will be treated for paraffin inclusion, and sections will be cut and stained with haematoxylin and eosin (HE). All histopathological examinations will be performed by the same blind pathologist. (Optical microscopy, 40° magnification). Several histological parameters will be evaluated.

Radiographic Analysis

Two radiologists will evaluate the x-rays blindly. Bone density will be assessed using the radiographic densitometric value of the control group as a reference. A visual scale will be used to evaluate all parameters, present or absent. Cone beam tomography will be used to obtain the radiographic images. Two rats from each subgroup will be housed on an acrylic platform, which will immobilize them for imaging. Osteolysis images will be taken in the sagittal, coronal and axial planes. The thickness of the sections is 1 mm and the distance between sections is also 1 mm for these reconstructions. The images of each rat will be analyzed separately by two radiologists.

Biological Analysis

Blood samples will be taken from the left ventricle for serum analysis four weeks after tooth extraction. The serum samples will be used to measure the C-terminal cross-linking telopeptide of type I collagen (CTX) and serum band 5 of tartrate-resistant acid phosphatase (TRACP-5b). CTX and TRACP-5b will be determined by enzyme-linked immunosorbent assays.

Judgement Criteria

Biochemical

Osteonecrosis will be defined by:

- Increase in serum concentration of resistant acid tartrate phosphatase
- Increase in serum C-Terminal Telopeptide concentration

Radiological

Osteonecrosis will be defined by:

- The thickening and disorganization of the spinal trabeculation,
- The thickening of the lamina dura,
- A border of peri-radicular osteosclerosis
- Thickening of the external cortex and dental spicules

Histological

Osteonecrosis will be defined by,

- 8 to 10 adjacent empty gaps in the alveolar bone, corresponding to necrotic foci.
- The presence of osteoclasts: arithmetic mean of their number in 3 different fields (40×),
- A visual scale from 1 to 5 (1: no vessels, 5: duplication of the vascular surface compared to this area in controls),
- The degree of alveolar remodelling (after tooth extraction): a scale has been established from 1 to 5 (1: no bone resorption, 5: 100% of the alveolar bone surface resorbed and replaced by fibrous tissue).

Statistical Analysis of the Data

Qualitative variables will be analyzed using the Pearson Chi-Square test and the Fisher exact test. Quantitative variables will be analyzed using a non-parametric KruskaleWallis test. A statistical significance level of 0.05 will be used. Data analyses will be performed using SPSS v. 11.5.

Ethical Considerations

The study protocol will be submitted to the Institutional Committee of Ethics and Research of the Faculty of Medicine and Biomedical Sciences. The study will be conducted in accordance with the European Union Directives on the use of experimental animals [27].

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