

Original article

Bone quality and osteonecrosis of the jaw induced by bisphosphonates

Julie Hémar^{1,2,3,*}, Thierry Sauvigné², Anne-Gaëlle Bodard⁴, Georges Boivin³

¹ School of dental Medecine, University, Lyon, France

² Department of Stomatology, Centre hospitalier Lyon-Sud, Hospices civils, Lyon, France

³ INSERM UMR 1033, Team bone quality and biological markers, Lyon, France

⁴ Department of Odontology, Centre régional de Lutte contre le Cancer Léon Bérard, Lyon, France

(Received 4 October 2011, accepted 24 October 2011)

Key words:

jaw / osteonecrosis /
bisphosphonates /
microradiography /
hardness tests

Abstract – Osteonecrosis of the jaw (ONJ) is a complication of long-term bisphosphonates therapy whose etiology and pathogenesis are not fully understood. Our purpose was to assess the quality of bone in the jaws of control subjects, and osteoradionecrosis (ORN) and ONJ patients.

Materials and methods: six jaw bone samples were taken from control patients. Eight jaw bone samples in patients with osteonecrosis (2 ORN and 6 ONJ) were also taken during conservative surgery. Histology was used to analyze cellular composition, microarchitecture and static variables of bone formation and resorption. Bone quality was also assessed using Vickers microindentation (measurement of microhardness) and quantitative microradiography [measurement of the mean degree of mineralization of bone (DMB) and of the heterogeneity index (HI) of the distribution of the mineralization].

Results: compared to controls, the osteonecrotic bone was hypovascularized, eroded, poor in osteoblasts and contained numerous empty periosteocytic lacunae. DMB (control group $1.14 \pm 0.13 \text{ g.cm}^{-3}$, ORN $1.25 \pm 0.14 \text{ g.cm}^{-3}$ and ONJ $1.11 \pm 0.12 \text{ g.cm}^{-3}$) and HI (control group $0.18 \pm 0.08 \text{ g.cm}^{-3}$, ORN $0.17 \pm 0.06 \text{ g.cm}^{-3}$ and ONJ $0.24 \pm 0.08 \text{ g.cm}^{-3}$) in osteoradionecrosis and ONJ samples were not significantly different from controls. Hypermineralization was never observed in ONJ. Bone microhardness after osteoradionecrosis ($50.04 \pm 4.34 \text{ kg.mm}^{-2}$) and ONJ ($47.48 \pm 4.10 \text{ kg.mm}^{-2}$) was slightly decreased compared to controls ($53.52 \pm 6.46 \text{ kg.mm}^{-2}$).

In conclusion, bisphosphonates-induced ONJ was neither associated with modifications of secondary bone mineralization, nor changes in bone microhardness. Bone vessels and cellular changes appeared to play a major role in the development of ONJ.

Mots clés :

maxillaires /
ostéonécrose /
bisphosphonates /
microradiographie /
tests de dureté

Résumé – Qualité osseuse et ostéonécrose des maxillaires due aux bisphosphonates. Les bisphosphonates (BP) sont de puissants inhibiteurs de la résorption osseuse utilisés dans le traitement de nombreuses pathologies comme les cancers et l'ostéoporose. L'ostéonécrose des maxillaires (ONM) est une complication du traitement au long cours par les BP. L'étiologie et la pathogénie de l'ONM induite par les BP ne sont pas complètement élucidées. En effet, seules des hypothèses ont été avancées. Notre objectif est d'évaluer et de comparer la qualité osseuse des maxillaires chez des sujets témoins, avec ostéoradionécrose (ORN) et avec ONM induite par les BP.

Matériel et méthodes : six échantillons osseux ont été prélevés chez des sujets témoins, dont quatre lors de la régularisation de crête osseuse après des avulsions dentaires, un lors de l'ablation d'un torus mandibulaire et un après une fracture. Huit échantillons d'ostéonécrose (deux dans le groupe ORN et six dans le groupe ONM) ont été prélevés lors d'interventions chirurgicales conservatrices (séquestrectomie et curetage à minima). Dans le groupe ONM, cinq patients ont été traités par zolédronate et une patiente par pamidronate puis zolédronate et clodronate. Tous les échantillons ont été étudiés avec les techniques histologiques afin d'analyser la composition cellulaire, la microarchitecture et les paramètres statiques de formation et de résorption du tissu osseux. L'évaluation de la qualité osseuse des échantillons a été effectuée par microdurimétrie Vickers et microradiographie quantitative.

* Correspondence: julie.hemar@wanadoo.fr

Résultats : Les observations histologiques et microradiographiques des coupes ORN et ONM mettent en évidence de nombreuses et larges surfaces érodées, par opposition aux coupes témoins. Les zones ostéonécrotiques d'ORN et d'ONM sont caractérisées par une hypovascularisation, mais aussi par une raréfaction des ostéoblastes, des ostéoclastes et des ostéocytes, par rapport aux échantillons témoins, avec parfois des zones dépourvues d'éléments cellulaires, caractéristiques de zones de tissu osseux non vital.

Le degré moyen de minéralisation (groupes témoin $1,15 \pm 0,12 \text{ g.cm}^{-3}$, ORN $1,25 \pm 0,14 \text{ g.cm}^{-3}$ et ONM $1,13 \pm 0,12 \text{ g.cm}^{-3}$) et l'index d'hétérogénéité (groupes témoin $0,19 \pm 0,07 \text{ g.cm}^{-3}$, ORN $0,17 \pm 0,06 \text{ g.cm}^{-3}$ et ONM $0,23 \pm 0,07 \text{ g.cm}^{-3}$) sont semblables dans les trois groupes.

La microdureté moyenne de l'os des échantillons ORN ($50,04 \pm 4,34 \text{ kg.mm}^{-2}$) et ONM ($48,03 \pm 3,90 \text{ kg.mm}^{-2}$) est un peu diminuée par rapport à celle des témoins ($53,48 \pm 5,78 \text{ kg.mm}^{-2}$).

Toutes ces valeurs sont compatibles avec des valeurs normales. Aucune hyperminéralisation n'est observée dans le tissu osseux des ONM.

Conclusion : l'ONM induite par les BP n'est pas associée à une modification de la minéralisation secondaire de l'os, ni à une modification de sa microdureté. Les changements vasculaires et cellulaires dans le tissu osseux semblent donc jouer un rôle primordial dans le développement de l'ONM.

Osteonecrosis of the jaw (ONJ) is a complication of the use of bisphosphonates (BP) and a painful condition characterized by bone necrosis in the oral cavity commonly associated with localized swelling [1]. ONJ is becoming more frequent due to the expanding indications for BP-therapy.

BP inhibit both bone resorption and bone remodelling [2] and they have additional antiangiogenic [3], immunomodulator [4] and direct antitumor [5] effects. However, because of their anti-catabolic effect, the precise mechanisms of action of BP are not fully elucidated.

The medical indications for BP are related to their mechanisms of action. This explains why they are widely used not only for osteoporosis, but also for the treatment of all pathological conditions characterised by increased bone resorption, such as Paget's disease of bone, osteolytic bone metastases (in patients with breast or prostate cancer), myeloma, hypercalcemia of malignancy and fibrous dysplasia of bone [6].

Because BP are not metabolised by the body, they are retained in bone long after the end of the treatment [7].

"Bone quality" encompasses a number of bone tissue characteristics contributing to the overall integrity of bone and to its mechanical resistance to fracture [8], such as bone mass, geometry and microarchitecture, bone tissue mineralization, quality of collagen and apatite crystal structure, and presence of microcracks [8]. All these properties are influenced by the rate of bone remodeling [9].

BP-induced ONJ refers to a condition characterized by prolonged (more than 8 weeks) exposure of bone in the mandible or maxilla in a patient treated with BP and without history of radiation therapy to the jaws [10]. ONJ results from bone exposure in the oral cavity with subsequent necrosis, and can either appear spontaneously or, more often, after dental procedures or traumatic injuries [11–14]. ONJ can affect both jaws, but lesions are more frequently seen in the mandible [2,7].

The true incidence of BP-induced ONJ is unknown and depends on the type of BP used, the route of administration,

the time of exposure, the cumulative dose and the indication of treatment [13,15]. The etiology and pathogenesis of BP-associated ONJ are poorly understood. Various hypotheses have been proposed, such as an antiangiogenic effect of the drug [16], infections, and hypermineralization associated with bone weakening and microdamage [17]. The pathophysiologic mechanisms underlying ONJ need to be clarified. The purpose of the present study was to assess the quality of bone in jaws from control, osteoradionecrosis (ORN) and ONJ patients, in order to evaluate the role of bone quality in the pathophysiology of BP-associated ONJ. Only bone mineralization and microhardness were measured.

Material and methods

The present study was performed using jaw bone samples from six patients with BP-induced ONJ and two patients with ORN. All test samples were collected during conservative surgery. Six normal samples (four collected during bone regularization after tooth removal, one during resection of a mandibular torus and one after jaw bone fracture) were used as controls.

The diagnosis of ONJ was suspected by the presence of various clinical signs, such as pain, soft-tissue swelling, or exposed bone, especially after dental work. No control or ONJ patient had received radiotherapy to the head and neck. All had received detailed information on the objectives of the study and provided informed consent to participate.

The mean time of exposure to BP was 22 months (standard deviation, 13 months). In the ONJ group, five patients were treated with zoledronic acid and one with pamidronate followed by an association of zoledronic acid and clodronate.

Bone samples were preserved in 70% alcohol, dehydrated in absolute alcohol, then embedded in methyl methacrylate without prior decalcification [18]. Eight-micrometer-thick sections were cut with a tungsten carbide knife (Leica Polycut E microtome) for histology. Sections were stained with May-Grünwald Giemsa, solochrome cyanin R or a modified Goldner's

trichrome stain, whereas some were left unstained for observation of bone structure under polarized light. Cellular components, bone texture (lamellar or woven bone, marrow fibrosis) and histological characteristics were assessed qualitatively in all samples. Thick sections (about 150 μm) were cut from embedded bone samples with a precision diamond wire saw (Well, Escil, Chassieux, France), then progressively ground to a thickness of 100 μm and polished with a diamond paste (1 μm particle size). The thickness of the section was measured with an accuracy of 1 μm using a precision micrometer (Compac, Geneva, Switzerland). After ultrasonic cleaning, bone sections were microradiographed [19–21] using a PW 1830/40 X-ray diffractometer equipped with a PW 2273/20 diffraction tube (Philips, Limeuil-Brévannes, France) operating at 25 kV and 25 mA and nickel-filtered copper $K\alpha$ radiation. A green-sensitive, high-resolution Geola VPR-M film was exposed for 20 min (Slavich International Wholesale Office, Vilnius, Lithuania). For quantitative evaluation of X-ray absorption by the bone section, aluminum step-wedges were exposed with each microradiograph. The degree of mineralization of bone (DMB) was quantified using automatic software for gray level analysis (Morpho Expert and Mineralization, Explora Nova, La Rochelle, France). Microscopic images of the microradiograph were captured using a digital camera (actual resolution: 1600 \times 1200 pixels or 800 \times 600 after binning). After calibration using the aluminum reference system, automatic selection of the measured bone tissue regions and bone thresholding, gray level images were segmented into homogeneous regions. The values of the gray levels were obtained at pixel level (at magnification 2.5 \times , the size of the pixel was 2.82 μm). Finally, gray-level values were converted to DMB values with the construction of a calibration curve based on the measurements obtained for the aluminum step-wedge. DMB expressed in grams of mineral per cm^3 of bone ($\text{g}\cdot\text{cm}^{-3}$) was measured in total (cortical + cancellous) bone tissue. The main variables extracted from DMB measurements were the mean DMB and the mean index of heterogeneity in the distribution of DMB (HI) expressed as the mean width at half-maximum measured on individual DMB curves.

Microhardness was measured with a Micromet 5104 tester (Buehler, Lake Bluff, Illinois, USA) equipped with a Vickers indenter on the 100 μm -thick sections previously used for microradiography, then meticulously surfaced and polished with an alumina suspension (1 μm) [21]. If orientation of the blocks before sectioning was feasible, a cutting plane perpendicular to the Haversian canals of cortical bone was preferred. The microhardness tester was linked to a computer using an OmniMet software for calculation of microhardness and digital processing of the values measured. Impression sizes were adapted to the study of the intermediate level of bone organization, i.e., the bone structural unit (BSU). The formula for calculating the microhardness of bone tissue was

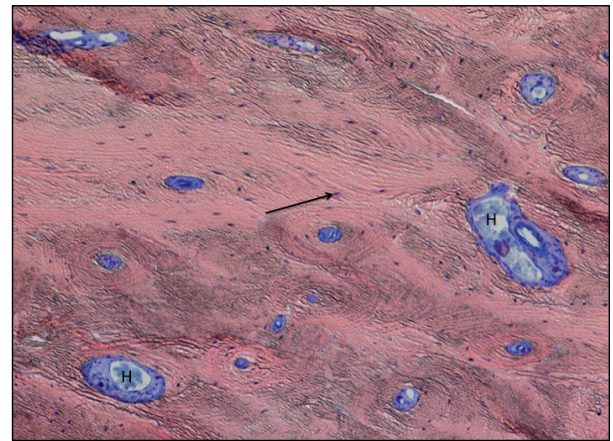


Fig. 1. Control specimen. Histological image of a bone section showing osteocytes in periosteocytic lacunae (arrow) and large capillaries in Haversian canals (H) (May-Grünwald Giemsa, original magnification 10 \times).

Fig. 1. Échantillon témoin. Coupe histologique osseuse mettant en évidence la présence d'ostéocytes dans les lacunes périostéocytaires (flèche) et de gros capillaires dans les canaux de Havers (H) (May-Grünwald Giemsa, objectif 10 \times).

$Hv = 1854.4 P/d^2$ (where Hv is Vickers microhardness expressed in $\text{kg}\cdot\text{mm}^{-2}$, P the test load expressed in g, and d the mean length of the two diagonals expressed in mm). In our laboratory, microhardness tests with Vickers indenter were performed at a load of 25 g for 10 s (intra and inter-observer coefficients of variation were $\leq 5\%$). Finally, global microhardness at the surface of bone samples was calculated as the mean of 10 randomly selected individual measurements separated by at least 500 μm . The distance between impression and the boundary of bone was at least 10 μm .

Results

Histological observations of all bone samples showed that the lamellar texture of new and old bone was maintained (Figs. 1–5). Osteoblasts were rare in ORN and ONJ specimens. No sign of morphologic osteoblastic activity was observed and osteoid seams were absent in all ORN and ONJ samples (Fig. 4), whereas a low remodelling activity (few osteoblasts and thin osteoid surfaces) was observed in controls (Fig. 2). Periosteocytic lacunae were almost always empty in ORN and ONJ samples (Fig. 3). Some bone areas were devoid of cellular elements, a characteristic of necrotic bone. Osteoclasts were also rare in ORN and ONJ specimens. However, contrary to controls, ORN and ONJ samples presented numerous eroded areas at the interface between bone and bone marrow (Figs. 4 and 5). Such “crenated bone” surfaces were particularly observable in ONJ specimens (Fig. 4).

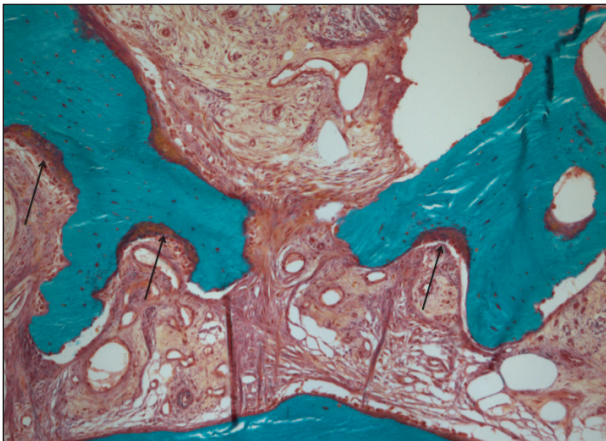


Fig. 2. Control specimen. Histological image of a bone section showing Howship resorption lacunae (arrows) with osteoblasts and osteoid tissue (modified Goldner's trichrome, original magnification 10x).

Fig. 2. Échantillon témoin. Coupe histologique osseuse mettant en évidence la présence de lacunes de Howship (flèches) avec des ostéoblastes et de la substance ostéoïde (trichrome de Goldner modifié, objectif 10x).

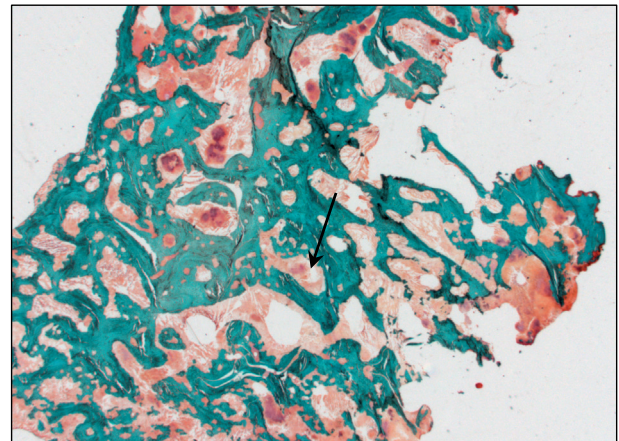


Fig. 4. Osteonecrosis of the jaw. Histological image of a bone section showing mainly inflammatory infiltration of vascular spaces with congested (arrow) and eroded areas without capillaries and osteoid (modified Goldner's trichrome, original magnification 5x).

Fig. 4. Ostéonécrose des maxillaires. Coupe histologique osseuse mettant en évidence des espaces vasculaires congestifs avec un infiltrat inflammatoire (flèche) et une surface très résorbée sans capillaires sanguins ni substance ostéoïde (trichrome de Goldner modifié, objectif 5x).

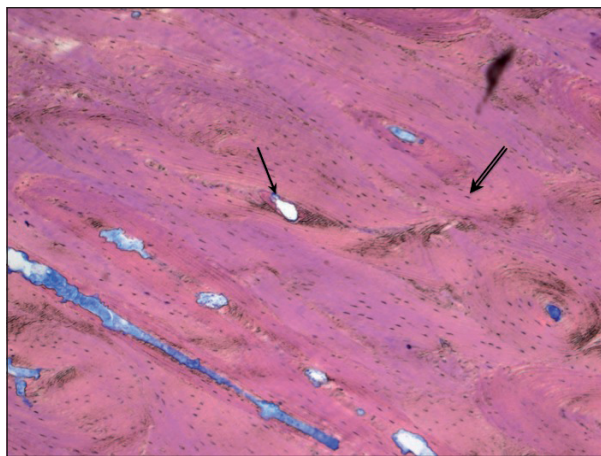


Fig. 3. Osteonecrosis of the jaw. Histological image of a bone section showing lamellar bone (osteons) with Haversian canals without capillaries (single arrow) and with empty periosteocytic lacunae (double arrow) (May-Grünwald Giemsa, original magnification 10x).

Fig. 3. Ostéonécrose des maxillaires. Coupe histologique osseuse montrant un os lamellaire (ostéons) avec des canaux de Havers dépourvus de capillaires sanguins (flèche simple) et des ostéocytes non-vitaux (cellules pycnotiques) dans les lacunes périostéocytaires (flèche double) (May-Grünwald Giemsa, objectif 10x).

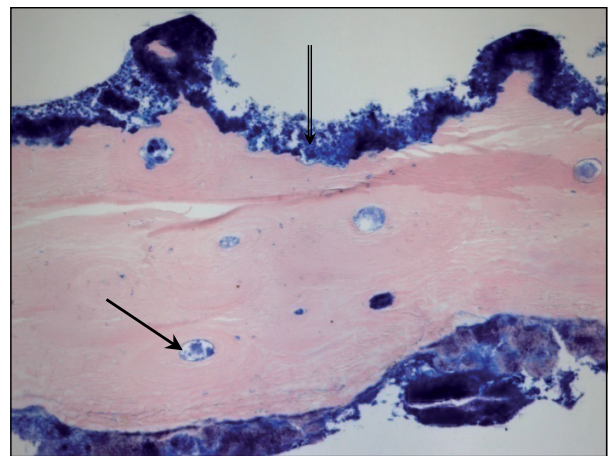


Fig. 5. Osteoradionecrosis of the jaw. Histological image of a bone section showing mainly inflammatory infiltration of vascular spaces with congested and eroded areas (May-Grünwald Giemsa, original magnification 5x).

Fig. 5. Ostéoradionécrose des maxillaires. Coupe histologique osseuse mettant en évidence un os très résorbé avec espaces vasculaires congestionnés par un infiltrat inflammatoire (flèche simple) et une surface très érodée (flèche double) (May-Grünwald Giemsa, objectif 5x).

While the Haversian canals of control samples contained capillaries and connective tissue (Figs. 1 and 2), ORN and ONJ specimens were poorly vascularized (Figs. 3–5).

Microradiographs of bone sections illustrated that DMB varied with the BSU: “young” BSU were less mineralized and more radiolucent than “old” BSU (interstitial bone) (Fig. 6).

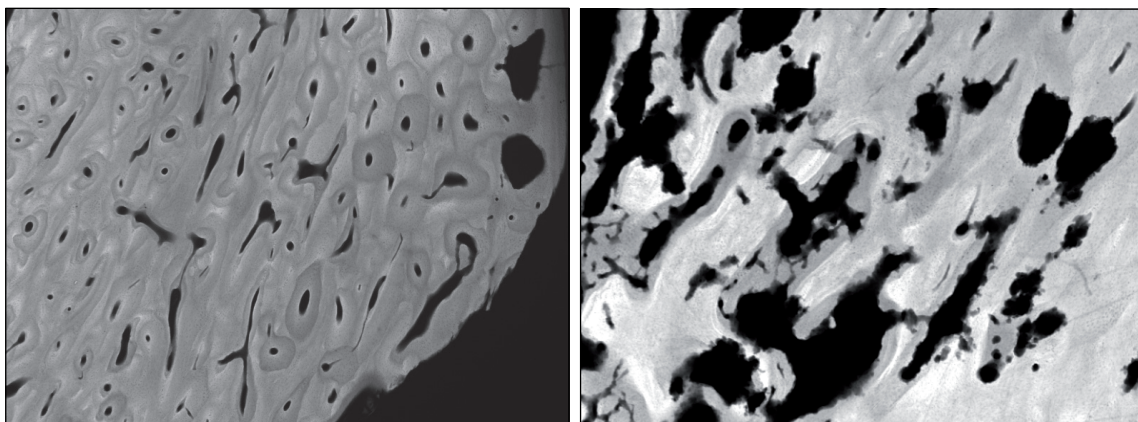


Fig. 6. Control specimen (on the left) and osteonecrosis of the jaw (on the right). Microradiographs of bone sections illustrating the heterogeneity of the mineralization in different Bone Structural Units (BSU). Mineral density appears more homogeneous in ONJ samples than in controls (original magnification 2.5×).

Fig. 6. Échantillon témoin (à gauche) et ostéonécrose des maxillaires (à droite). Microradiographies de coupes osseuses mettant en évidence l'hétérogénéité de la minéralisation des unités de remodelage (Bone Structural Units = BSU). La minéralisation est distribuée de façon plus homogène dans l'échantillon ONM que dans l'échantillon témoin (objectif 2,5×).

Table I. Variables reflecting bone mineralization and microhardness in the three groups of bone samples studied (ORN: osteoradionecrosis, ONJ: osteonecrosis of the jaw, DMB: degree of mineralization of bone, HI: heterogeneity index of mineralization, Hv: bone microhardness). *Tableau I. Paramètres reflétant la minéralisation et la microdureté osseuses dans les trois groupes d'échantillons osseux étudiés (ORN : ostéoradionécrose, ONJ : ostéonécrose des maxillaires, DMB : degré de minéralisation osseuse, HI: index d'hétérogénéité de la minéralisation, Hv : microdureté osseuse).*

	Control group	ORN group	ONJ group
DMB (g.cm ⁻³)	1.15 ± 0.12*	1.25 ± 0.14	1.13 ± 0.12
HI (g.cm ⁻³)	0.19 ± 0.07	0.17 ± 0.06	0.23 ± 0.07
Hv (kg.mm ⁻²)	53.48 ± 5.78	50.04 ± 4.34	48.03 ± 3.90

* mean ± standard deviation.

* moyenne ± écart type.

Variations of DMB and HI were not different between controls, ORN and ONJ samples. Bone microhardness in ORN and ONJ samples was similar, and slightly lower than in controls (Table I).

Discussion

The etiology and pathogenesis of ONJ are not clear. Several hypotheses have been proposed to explain the occurrence of ONJ in patients undergoing long-term BP therapy. However, the lack of microbiological data on the bone samples studied did not allow to discuss the infectious hypothesis [17].

BP-induced ONJ has been compared to a combination of osteomyelitis and osteopetrosis [22,23] because jaw bone exposure is a rare complication of osteopetrosis, with the same clinical signs as in BP-induced ONJ. Osteopetrosis is characterized by an excess of bone mass due to defective bone resorption, pathological fractures, small medullary cavities and narrowed bone channels for blood supply [24]. BP-induced ONJ is sometimes presented as a form of chemically induced osteopetrosis [22]. However, in osteopetrosis, high bone mass and hypermineralization provoke not only the reduction in size of the marrow cavity, but also the obliteration of Haversian canals containing blood vessels, which may induce blood vessel compression and decreased blood supply to bone [24,25]. Our histological observations revealed that the size of Haversian canals in ONJ samples was normal. The mineralization process consisted in a primary deposition of mineral substance on the calcification front, followed by a slow, progressive increase in mineral deposition, called secondary mineralization [9]. Secondary mineralization increased with bone age [21] and with the decrease of bone remodelling due to anti-resorptive therapy [20]. BP treatment leads to the occurrence of more mature bone with many old BSU that have achieved complete mineralization [20]. This full mineralization of old BSU corresponds to a complete secondary physiological mineralization rather than to a "hypermineralization" which would imply the presence of an excessive amount of mineral in the same volume of bone tissue [26]. DMB and HI measurements in control, ORN and ONJ samples were similar to values previously reported in normal bone [19,21]. In our ONJ patients, signs of hypermineralization were never observed. Therefore, the comparison between ONJ and osteopetrosis, and the hypothesis of

a hypermineralization associated with bone weakening [17] seems unlikely. Our results suggest that the development of BP-induced ONJ is not due to a hypermineralization of the bone matrix.

BP-induced ONJ has often been compared to ORN [11,27]. The present data suggest some similarities between ORN and ONJ samples compared to controls: hypovascularization and hypocellularity of bone tissue, signs of former important resorption not followed by bone formation.

As regard the antiangiogenic effect hypothesis [16], BP inhibit both capillary neoangiogenesis and endothelial proliferation, thus leading to loss of blood vessels and avascular osteonecrosis. Hypovascularization may lead to bone cell death and to a lack of osteoprogenitor cells causing low bone remodelling [16]. In our ORN and ONJ samples, both poor vascularization of necrotic bone and a lack of capillaries in Haversian canals were observed.

ONJ could be due to the cessation of bone remodelling and bone turnover due to the antiresorptive effect of BP. Actually, when osteoclasts are inhibited, bone formation is indirectly altered [17]. At jaw bone level, daily physiological mastication causes bone microdamage [10,28,29] that is normally repaired by targeted bone remodelling under the control of osteocytes [30]. However, osteocytes, osteoblasts and osteoclasts were rarely identified in our ORN and ONJ samples. In the absence of osteoclastic bone resorption, the removal of old bone followed by the formation of new bone cannot occur [10] and necrotic bone accumulates.

These results are supportive of hypotheses suggesting that a combined lack of vascular perfusion and decreased bone remodelling could cause the accumulation of bone microdamage and consequently osteonecrosis [6].

Our study has some limitations. Since conservative treatment must be as long as possible, the number of bone samples available for analysis was limited and statistical analysis was impossible. Samples were not calibrated and not taken from the same anatomical site, which made comparisons difficult. Finally, the absence of tetracycline labelling prevented the evaluation of bone formation.

To conclude, the treatment of BP-induced ONJ was neither associated with modifications of the secondary mineralization of bone, nor with variations of bone microhardness. Vascular and cellular changes in bone tissue appeared to play a major role in the development of ONJ.

Competing interests: none

Acknowledgments. The authors thank Catherine Simi, Delphine Farlay, Yohann Bala and Jean-Paul Roux for expert technical assistance. This work was financially supported by the INSERM and the School of Dental Medicine of the University of Lyon.

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