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INVITED MEDICAL REVIEW

Bisphosphonate-related osteonecrosis: genetic and acquired risk factors

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The objectives of this study were to review epidemiological, clinical and biological aspects associated with the development of bisphosphonate-related osteonecrosis of the jaw (BRONJ) in multiple myeloma (MM) patients, with special emphasis on the genetic aspects. A detailed review of previously described risk factors as well as recent genetic findings mostly comprises this work. The most recent meeting abstracts and relevant articles published in journals covered by the Science Citation Index and Medline are also examined. The review pays special attention to the genetic component of BRONJ. A total of 15 series and 14 guidelines or revisions were selected to fit the aims of the review. Gene variability was reviewed in depth to give a clinical illustration on the genetic aspects of BRONJ. Crude prevalence and 5-year cumulative incidence were considered as the most important end points for predictive purposes. Several acquired factors were recognized as predictors for BRONJ in MM, especially intravenous bisphosphonates, dental trauma and advanced age. Among genetic factors, polymorphisms on *CYP2C8* gene arise as a promising risk factor. Bisphosphonate-related osteonecrosis of the jaw can be predicted with a conjunction of genetic and environmental risk factors.

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Introduction

In 1996, the first evidence supporting the clear benefits of intravenous bisphosphonates (BPs) in the therapy of bone disease associated with multiple myeloma (MM) was published (Berenson *et al*, 1996). Until that time, the only chance to counteract the devastating effects of this disease was to wait for the response of antineoplastic therapy and its potentially favourable impact on the bones. However, this involves the occurrence of more than 60% of skeletal events during the treatment of such patients (Berenson *et al*, 1998), including pathological fractures, vertebrae collapse, hypercalcaemia and requirements for radiotherapy or surgery. The use of intravenous BPs has now completely changed this scenario and currently the incidence of skeletal-related events (SRE) has decreased by 50% or more (Badros *et al*, 2008). Nevertheless, with the use of these drugs, relevant new complications have emerged as important clinical problems for such patients (Terpos *et al*, 2009). Among them, bisphosphonate-related osteonecrosis of the jaw (BRONJ) is the most disturbing and serious complication related to the use of BPs, although its frequency cannot be considered high. Accordingly, the identification of risk factors potentially associated with the development of BRONJ is a critical point in the management of patients with malignancies associated with bone disease.

In this paper, we review the incidence of BRONJ among patients with multiple myeloma bone disease, and the clinical and biological characteristics that are associated with this complication and that could be used as predictors for better clinical management, with special emphasis on the genetic aspects of such characteristics.

Clinical relevance of bisphosphonates

Currently, a broad range of BPs with differences in their potency, efficacy, dosing and administration as well as their approved indications is available (Kyle *et al*, 2007; Terpos *et al*, 2009). BPs inhibit osteoclast-mediated bone resorption and are the standard of care for tumour-associated hypercalcaemia (Russell *et al*,

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2007). Thus, BPs have been used for more than 15 years to reduce bone pain, to improve the quality of life and to delay skeletal events such as pathological fractures (Stewart, 2005; Berenson *et al*, 2006).

A prominent manifestation of MM is bone destruction, which appears in 90% of patients. Apart from the associated pain, this leads to complications known as SRE: pathologic fractures or the development spinal cord collapse/compression, the need for surgery or therapeutic radiation therapy due to bone involvement and hypercalcaemia of malignancy (Berenson *et al*, 1996). The use of BPs is highly recommended for these patients, as these drugs have been shown to afford relevant clinical benefits (Esteve and Roodman, 2007; Kyle *et al*, 2007; Terpos *et al*, 2009).

Bisphosphonates are synthetic analogues of pyrophosphate that bind avidly to divalent metal ions such as Ca^{2+}, especially at sites of active bone remodelling, resulting in a strong anchoring of BPs to bone mineral surfaces *in vivo* (Masarachia *et al*, 1996). There are two groups of BPs, depending on the mechanism of action: generic non-nitrogen-containing compounds (clodronate, etidronate), which inhibit bone resorption by inducing osteoclast apoptosis, and the more recently developed nitrogen-containing compounds (pamidronate, zoledroniz acid), with much greater potency. These latter substances inhibit the osteoclast mevalonate pathway by decreasing the isoprenylation of proteins such as Ras and other GTPases (Zhang and Casey, 1996). These signalling proteins regulate a variety of cell processes necessary for osteoclast function, including cytoskeleton arrangement, membrane ruffling, the trafficking of intracellular vesicles and cell survival (Coxon and Rogers, 2003).

When added to chemotherapy in patients with newly diagnosed myeloma, oral etidronate has been shown to be ineffective (Belch *et al*, 1991), whereas oral clodronate showed small benefits (Delmas *et al*, 1982; Merlini *et al*, 1990; Lahtinen *et al*, 1992; Clemens *et al*, 1993; McCloskey *et al*, 1998). Intravenous administration of nitrogen-containing BPs appears to be associated with higher efficacy in terms of pain reduction, delay of SRE and improvement in the quality of life in both MM and breast cancer patients (Berenson *et al*, 1996, 1998; Rosen *et al*, 2001, 2003).

Nevertheless, these unquestionable benefits are accompanied by undesirable events. Initially, the overall adverse effect profile of BPs was considered to be relatively mild, consisting merely of pyrexia, a deterioration of renal function and hypocalcaemia (Bilezikian, 2006). However, the positive benefits, safety and efficacy of new therapies are often emphasized and, occasionally, they overshadow and delay the appreciation of potential side effects. This is the case of intravenous BPs, which were widely accepted in 2002 (Berenson *et al*, 2002). However, since 2003, an increasing number of reports have highlighted the probable association between the development of so-called osteonecrosis of the jaw in multiple myeloma and breast cancer and the use of pamidronate and, especially, zoledronic acid (Marx, 2003; Ruggiero *et al*, 2004; Durie *et al*, 2005; Edwards *et al*, 2008). This resulted in a revision by the US Food and Drug Administration of the label for zoledronic acid and pamidronate.

Although the exact mechanism of BRONJ has not yet been determined, several hypotheses have been advanced. The condition has been attributed to a reduction or loss in vascular supply, in particular with zoledronic acid (with potent antiangiogenic properties) that could contribute to the apparent ischemic changes noted in the affected jaw bones (Wood *et al*, 2002; Santini *et al*, 2003). However, impaired bone resorption associated with specific circumstances in the oral cavity, such as bone remodelling in the maxilla, frequent trauma and the presence of oral saprophytic flora may also contribute (Bamias *et al*, 2005). In addition, other causes such as genetic factors could be involved.

Clinical presentation of BRONJ

Currently, ONJ is defined as an area of exposed bone in the maxillofacial region in the absence of radiotherapy that has not healed within 8 weeks after identification by a healthcare worker (Khosla *et al*, 2007). The differential diagnosis of ONJ should exclude other common clinical conditions such as alveolar osteitis, sinusitis, gingivitis and periodontal disease.

The signs and symptoms observed before the clinical detection of ONJ may include pain, mucosal swelling, erythema, ulceration and loose teeth. It has been observed that the lesions occur more frequently in the mandible than in the maxilla (2:1 ratio). The size of the lesion varies, ranging from a non-healing extraction site to exposure and necrosis of large sections of the jawbone, and the presence of bacteria without evidence of bacterial invasion as a common finding (Ruggiero and Drew, 2007).

Frequency and risk factors

The true frequency of BRONJ is hard to determine as the use of oral and intravenous BPs is widespread, and BRONJ is probably an under-recognized entity. The current difficulty in establishing accurate data concerning its incidence stems from several factors. First, the incidence of ONJ in the general population not exposed to BPs is unknown. Second, the incidence of ONJ is very different in patients receiving oral BPs for different conditions such as osteoporosis or Paget's disease compared with patients receiving high doses of intravenous nitrogen-containing BPs for the management of bone disease secondary to a malignant disorder (Khosla *et al*, 2007). Thus, the estimated prevalence of BRONJ in patients receiving intravenous BPs for malignant diseases ranges from 0.8% to 12% (AAoOaMS, 2007; Pozzi *et al*, 2007; Hoff *et al*, 2008), although it has been reduced by implementing preventative measures (Dimopoulos *et al*, 2009). In our series of 675 MM patients receiving BP therapy with either pamidronate ($N = 452$), zoledronic acid ($N = 158$) or both sequentially ($N = 65$), with a median follow-up of 64 months, 24 of them (3.6%)

developed ONJ and 651 did not, providing a cumulative incidence of 5.6% at 5 years (Sarasquete *et al*, 2008).

This situation probably reflects the diversity of patients that could be affected by the onset of this complication. Such differences constitute risk factors that should be taken into consideration in clinical decision-making during patient management. Although no extensive studies have been performed to identify such risk factors, there is now some relevant evidence that allows two groups of risk factors for BRONJ to be distinguished.

Environmental risk factors

As previously mentioned, the potential mechanism of how BPs could induce ONJ remains unclear. Several retrospective clinical studies have identified potential predisposing risk factors: use of intravenous *vs* oral BPs (Bilezikian, 2006), concomitant use of chemotherapy (Schwartz, 1982), treatment with glucocorticoids (Wang *et al*, 2003; AAoOaMS, 2007) or thalidomide (Zervas *et al*, 2006), length of exposure to BP treatment (Badros *et al*, 2006; Dimopoulos *et al*, 2006; Bagan *et al*, 2009), the presence of co-morbid conditions such as obesity, alcohol and/or tobacco abuse (Wessel *et al*, 2008) and pre-existing dental or periodontal disease (Marx *et al*, 2005; Badros *et al*, 2006; Khosla *et al*, 2007). Among them, dental trauma, such as a dental extraction, is the most common immediate precipitating risk factor (Badros *et al*, 2006; Mehrotra and Ruggiero, 2006). Furthermore, the risk of BRONJ increases with each additional year of follow-up (57% increase per year) and with increasing patient age (9% with each decade of life) (Badros *et al*, 2006; Bagan *et al*, 2006; Khosla *et al*, 2007).

Despite the foregoing, a multidisciplinary panel of experts that met in 2007 reviewed all pertinent data published on BP-associated ONJ and recognized that the evidence of environmental risk factors predisposing individuals to this complication was not very strong (Hirschhorn and Daly, 2005).

Genetic risk factors

As only a minority of BPs users develop bone ONJ, it is conceivable that individual genetic variations in drug metabolism or skeletal homeostasis may confer susceptibility or resistance in developing the complication. Thus, like many other complex traits, ONJ could be caused by a combination of environmental and genetic risk factors. Genetic susceptibility may be conferred by multiple genes with small variations (De Gobbi *et al*, 2006). Identifying these relevant genes has been difficult, in part because each causative gene only makes a small contribution to the overall heritability. Two approaches, linkage analyses and association studies, are commonly used to identify susceptibility genes involved in tumourigenesis. However, association studies that require the genotyping of a large number of polymorphisms (usually single nucleotide polymorphism SNPs) across the genome are more powerful for the identification of genes contributing to the risk of common complex disease (Raje *et al*, 2008).

Accordingly, genome-wide association studies, which allow the identification of thousands of SNPs in a given population, and comparisons of the frequencies of alleles or genotypes of a particular variant between disease cases and controls, may help to identify many variants that could contribute to the development of ONJ. We carried out a genome-wide association study, typing half a million SNPs in two groups of MM patients who had received the same anti-myeloma therapy, but who featured a significant difference: the presence or absence of ONJ. There were 22 cases (MM with ONJ) and 65 controls (MM without ONJ) matched for age, gender and ethnicity. All patients had received the same chemotherapeutic protocol (GEM-2000) (Lahuerta *et al*, 2008) and BP therapy with intravenous nitrogen-containing BPs. Among several SNPs related to the presence of BRONJ, we identified four, showing a statistically significant association with ONJ within the same gene: rs1934951, rs1934980, rs1341162 and rs17110453. These four SNPs map within cytochrome P450, subfamily 2C polypeptide 8 gene (*CYP2C8*), which spans a 31-kb region located at the cytochrome P450 gene cluster of chromosome 10q23.

These findings demonstrate that *CYP2C8* gene diversity influences the likelihood of the development of ONJ in MM patients receiving BP therapy. It is not yet known how this effect occurs, as no functional studies comparing different gene conformations are available. However, it is well known that the presence of certain SNPs can favour or reduce *CYP2C8* gene expression in different ways (Hichiya *et al*, 2005; Hilli *et al*, 2007), which could include alterations of the promoter region (Li *et al*, 2007), splicing site modifications (Ingelman-Sundberg *et al*, 2007) and intronic microRNA variations (Ingelman-Sundberg *et al*, 2007). As the CYP2C8 protein is mainly expressed in the liver, where it participates in the metabolism of many drugs, this could explain how it might be able to promote differences in the final effect of a given molecule (Fleming, 2007). Nevertheless, as BPs do not seem to undergo any physico-chemical modifications, polymorphisms on *CYP2C8* would probably not be involved in the appearance of BRONJ in this way. Despite this, there are other biological pathways affected by the *CYP2C8* gene (Capdevila *et al*, 2000). CYP2C8 metabolizes arachidonic acid to epoxyeicosatrienoic acids (Viccica *et al*, 2007), which play a key role in the regulation of vascular tone and cardiovascular homeostasis (Luckman *et al*, 1998). As BRONJ is an avascular necrosis of the jawbone, alterations of this pathway due to a variant of *CYP2C8* could make the development of osteonecrosis more likely. This mechanism would be synergistic with other potential mechanisms, such as the direct effect of nitrogen-containing BPs on oral mucosal cells. Landesberg *et al* (2008) have shown *in vitro* that at clinically relevant doses, the pretreatment of murine oral keratinocytes with pamidronate inhibits proliferation and wound healing through mechanisms not dependent on apoptosis.

The other pathway possibly affected by CYP2C8 is the 3-hydroxy-3-methyl-glutaryl-CoA reductase

pathway. This key metabolic cascade for cholesterol synthesis seems to play a relevant role in osteoblastic differentiation (Bertoldo *et al*, 2007), a cell activity directly involved in bone remodelling. In addition, BPs inhibit farnesyl pyrophosphate synthase, which directly kills osteoclasts (Landesberg *et al*, 2008; Scheper *et al*, 2009), the second cell lineage involved in bone remodelling. The maxilla and mandible are characterized by very high bone remodelling activity (Ruggiero *et al*, 2006), a physiological mechanism whose delicate and continuous physiological balance is a mandatory requirement for recovery from injuries caused by tooth extractions. Accordingly, any disturbance in the balance between the destruction and formation of bone resulting from the interaction between BPs and *CYP2C8* polymorphisms will contribute to the development of ONJ in these patients.

Regardless of the mechanism of action, some polymorphic variations in *CYP2C8* are associated with a high risk of BRONJ. Thus, SNP rs1934951 (C/T, intron 8) was significantly associated with ONJ when the T allele was present, as this occurred in 48% of cases, whereas it only occurred in 12% of controls ($P = 0.000001$, OR 12.75, 95% CI: 3.7–43.5) This meant that patients carrying the T allele in this SNP were 12.5 times more likely to develop an episode of ONJ than the other cases. This SNP showed the strongest association with ONJ, but other SNPs also did so, although with a higher *P*-value: SNPs rs1934980 (A/G), rs1341162 (C/T) and rs17110453 (A/C) ($0.000004 < P < 0.00002$). Haplotyping analysis afforded two main haplotypes with three SNPs (rs1934951, rs1934980 and rs1341162) closely related to BRONJ: CAC (in 50% *vs* 83% of cases and controls, respectively) and TGT (in 45% *vs* 10%, respectively).

All these findings together suggest the existence of genetic risk factors favouring BRONJ development in certain specific subgroups of patients, and in this case those with specific *CYP2C8* genetic polymorphisms. Lehrer *et al* (2009) have proposed that other genetic conditions could be associated with BRONJ, in particular those related to gene and protein profiles that could result in altered bone remodelling in patients with ONJ. They suggested matrix metalloproteinase 2 as a candidate gene for these relationships. However, this hypothesis was merely based on theoretical aspects (von Ahsen and Oellerich, 2004).

Conclusions and further recommendations

Bisphosphonate-related osteonecrosis of the jaw is a relevant clinical problem in the management of patients suffering from malignant diseases with associated bone pathology. This has resulted in the development of some concern about the use of BPs to treat and prevent the skeletal complications of this disease.

However, BPs are very efficient drugs that have reduced the risk of SRE in patients with bone disease associated with malignancy by 50%, if not more. Consequently, renouncing to these drugs would place patients at a very high risk of developing advanced bone disease, with devastating final effects. In addition, BRONJ is now well defined and there are a number of risk factors to predict the possibility of a given patient developing this complication. Accordingly, a number of preventive actions can be taken, with very good results (Badros *et al*, 2008). In this sense, it is crucial to identify which patients are at high risk of suffering BRONJ to implement measures to minimize the clinical problems deriving from the problem.

There are several acquired variables that increase the risk of the development of BRONJ: dental extractions, pre-existing dental or periodontal disease, chemotherapy, glucocorticoids and thalidomide, long-term exposure to BP and the presence of co-morbid conditions such as obesity, alcohol and/or tobacco abuse. In addition, there are some genetic variations that are strongly associated with this complication. Among them, the presence of the T allele in the SNP rs1934951 located in the *CYP2C8* gene increases the risk of the development of BRONJ in MM patients treated with chemotherapy and stem cell transplantation by 12.5-fold.

Accordingly, it could be of value to perform all genetic studies to detect patients with a high risk of developing BRONJ to specifically avoid or reduce the use of BPs in them, or at least to carry out specific measures aimed at preventing BRONJ, or obtaining an early diagnosis of the disease, to reduce the problems caused by this complication when it occurs.

Future studies addressing the management of bone disease, including the refinement of genetic investigations, should attempt to confirm these findings in independent series, and they should also try to extend our knowledge about genetic events and their functionality in the mechanism of BRONJ development.

Author contributions

ME Sarasquete designed the study, performed the literature review and prepared the initial version of the paper. M González and JF San Miguel reviewed intermediate drafts and R García-Sanz did the final revision and gave the definitive approval for the submission. This work was partially financed by FIS-PI-06/1354 and FIS-CA-08/00212.

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