Platelet-rich therapies in the treatment of intravenous bisphosphonate-related osteonecrosis of the jaw: A report of 32 cases

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S U M M A R Y

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is an important complication in cancer patients taking intravenous BPs (BPs). In most cases, BRONJ is associated with an oral surgery procedure involving jaw bone. Currently, BRONJ management remains controversial, and there is no definitive standard of care for this disease. In fact, several articles in the recent literature discuss treatments that range from topical to surgical treatment, without definitive conclusion about treatment. A clinical study was conducted on 32 patients treated with i.v BPs for oncologic pathologies affected by BRONJ. The patients were treated by resection of the necrotic bone with primary closure of the mucosa over the bony defect using plasma rich in growth factors (PRGF). Orthopanoramic and computed tomography were performed before and after surgery. No intraoperative complications were observed, and all 32 cases were treated successfully. Our data on the use of PRGF demonstrate positive results for this surgical treatment. PRGF may enhance vascularization and regeneration of osseous and epithelial tissues.

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Introduction

Bisphosphonates (BPs) are a widely used class of drugs that are effective in the prevention and treatment of pathologies such as malignant hypercalcemia, Paget’s disease, postmenopausal osteoporosis, bone metastasis associated with solid tumors and multiple myeloma. These pathologies are characterized by metabolic imbalance involving high bone turnover and increased bone resorption resulting in skeletal-related events.1-4

The efficacy of nitrogen-containing bisphosphonates (NBP) is due to their ability to inhibit osteoclast-mediated bone resorption. Moreover, the effectiveness of BPs in reducing bone pain has been shown to consistently improve the prognosis and quality of life of patients. All of these reasons have led to the widespread use of these drugs.5

In 2003, Marx was the first to demonstrate that oncological patients who receive BPs occasionally manifest bisphosphonate-related osteonecrosis of the jaw (BRONJ).6 Currently, BRONJ is recognized as a significant complication related to the use of BPs, and more specialists are becoming aware of BRONJ due to the increasingly widespread use of these medications.

BRONJ is defined as an avascular area of necrotic bone in the maxillofacial area, with or without exposed bone, that has been evolving for more than eight weeks. BRONJ occurs in patients who have received and/or are receiving bisphosphonates without a previous history of irradiation in the maxillofacial region.7

It has been largely demonstrated that intravenous administration of BPs is an important risk factor for BRONJ. When compared to oral administration, intravenous administration has a greater infused dose.8

The onset of BRONJ is often subtle. If neglected, the lesion can progress and result in complications such as altered sensation in the affected area (e.g., the inferior alveolar nerve), oro-antral or oronasal communications, intraoral and extraoral fistulae and mandibular fractures.9,10

Currently, BRONJ management remains controversial, and there is no definitive standard of care for this disease. In fact, several articles in the recent literature discuss a range of topical to surgical treatment.11-15

The objective of our study is to find a surgical protocol that favours both bone and mucosal healing processes using PRGF. Platelet-enriched preparations represent a relatively new biotechnology for the stimulation and acceleration of tissue healing and bone regeneration. In 1999, Anitua was the first to introduce a new protocol for platelet gel preparation by producing plasma rich in growth factors (PRGF).16-18 PRGF seems to offer many advantages by allowing for the action of multiple growth factors and increasing tissue vascularization. In addition, PRGF is an autologous prod-
uct. Therefore, it is a biocompatible and safe product.\textsuperscript{19} The growth factors in PRGF promote angiogenesis and bone and mucosal healing.\textsuperscript{20}

The aim of this study was to evaluate the PRGF effectiveness on the surgical treatment of BRONJ.

Materials and methods

Thirty-two patients (22 female and 10 male) affected by BRONJ were included in a retrospective study. Twenty-four sites of BRONJ were located in the mandible and eight were located in the maxilla.

For inclusion in the study, the patients had to undergo surgical treatment. This patients did not have any other kind of treatment before surgical procedures. Among the whole BRONJ patients, we choose only lesions meeting Marx IIB classification,\textsuperscript{21} to have a very homogeneous sample. In all of the cases, BRONJ was clinically diagnosed and confirmed radiographically with TC\textsuperscript{22} (Fig. 1). BRONJ occurred in association with dental surgery, periodontal diseases and ill-fitting dentures. Recurrent BRONJ onset presenting subjects as well as zoledronic acid assuming osteoporotic patients were excluded.

As the study is completely retrospective, we decided to limit the observation in an old period of time, to have long control time. The observation period for the study started in June 2006 and ended in October 2010. Operations were performed from June 2006 to November 2006. The study was conducted in the Oral Surgery Department of the Dental School of the University of Torino, Italy. At the time of diagnosis, each patient was asked for a detailed history concerning the use, dose, frequency, and duration of therapy with BPs. No patient discontinued treatment with i.v BPs till the end of the observation period. The i.v. BPs that were administered were Zoledronic acid (Zometa\textsuperscript{a} Aclasta\textsuperscript{a}) or Pamidronate (Aredia\textsuperscript{a} Linoten\textsuperscript{a} Pamifos\textsuperscript{a}). Patients treated with Zoledronic acid preparations had a 4 mg infusion every 21 days, and those treated with Pamidronate had a monthly 90 mg infusion.

The characteristics of the patients and of BRONJ are listed in Table 1 and Table 2.

The local ethics committee approved the clinical protocol used for the study, and all of the patients that were enrolled in the study gave written informed consent.

A professional hygiene session was performed with each patient one week before surgery. Beginning the evening before surgery, each patient took amoxicillin at a dosage of one tablet every eight hours for a total of ten days.

Blood was obtained several minutes before starting surgery and prior to anesthesia administration.

![Figure 1 Ct scan shows the presence of a jaw injury.](image)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Some patients characteristics at the time of the diagnosis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Males 10 Females 22</td>
</tr>
<tr>
<td>Age</td>
<td>44–60 7 60–70 16 70–83 9</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>No 20 &lt;15/die 9 &gt;15/die 3</td>
</tr>
<tr>
<td>Primary disease</td>
<td>Prostatic carcinoma 6 breast carcinoma 5 multiple myeloma 14 Lung carcinoma 4 ovarian carcinoma 3</td>
</tr>
<tr>
<td>Drug prescribed</td>
<td>Zoledronic ccid 26 Pamidronate 6</td>
</tr>
<tr>
<td>Other medications</td>
<td>Steroids 11 Chemiotherapy 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Some BRONJ characteristics at the time of the diagnosis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of BRONJ</td>
<td>Mandible 24 Maxilla 8</td>
</tr>
<tr>
<td>Cause for BRONJ</td>
<td>Tooth extraction 17 ill-fitting dentures 7 Periodontal disease 8</td>
</tr>
<tr>
<td>Pain</td>
<td>* 20 – 12</td>
</tr>
<tr>
<td>Presence of pus</td>
<td>* 15 – 17</td>
</tr>
<tr>
<td>Exposed/necrotic bone</td>
<td>* 21 – 11</td>
</tr>
<tr>
<td>Oral fistulas</td>
<td>* 15 – 17</td>
</tr>
<tr>
<td>Treatment result</td>
<td>* 32 – 0</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>45–50 months 6 50–55 months 9 55–60 months 17</td>
</tr>
</tbody>
</table>

The PRGF used in this study were obtained by following the protocol described by Anitua. Ten to twenty milliliters of blood were drawn from the peripheral vein of each patient in the study using 5 mL tubes containing 3.8% trisodium citrate solution as an anticoagulant. The tubes were centrifuged at 1800 rpm for 8 min (PRGF System, BTI Biotechnology Institute, Milan, Italy) at room temperature. The blood was separated into the three basic components: red blood cells (at the bottom of the tube), PRGF (in the middle), and plasma poor in growth factors (PPGF). The fraction (0.5 mL) located immediately above the erythrocytes was collected from each tube and transferred to sterile tubes. Calcium chloride (50 $\mu$L) at 10% was added for 1 mL fraction of PRGF. After 15 to 20 min, a PRGF gel was formed. The time delay between PRGF gel formation and filling the bone defect was standardized at 5 to 10 min.\textsuperscript{23}

An alveolar nerve block infiltration was administered with local or regional anesthesia, depending on the dental arch, using 2% mepivacaine. Because mepivacaine does not contain epinephrine, it was used to prevent restriction of the blood supply. Intraligamentous and intrapapillary infiltrations were not done to avoid interrupting the healing process.

Surgery consisted in resecting all of the infected and necrotic bone. In cases where teeth were present, the teeth were extracted if they were less than 3 mm from BRONJ site. Resection margins were pre-surgically evaluated with a CT scan and clinically determined by the appearance of bleeding bone. All thin bone margins were rounded off with a piezoelectric device in order to avoid tissue overheating (Mectron Piezosurgery\textsuperscript{a} Device, Mectron Medical
Technology, Carasco, Italy). Osteoplasty and oxygenation of bleeding bone were also performed with a Piezosurgery® (Fig. 2, Fig. 3, Fig. 4).

The bone surfaces were covered by a PRGF fraction, and a membrane made up of a plasma fraction poor in growth factors was placed between the bone tissue and the mucosal flap to promote healing and prevent bleeding (Fig 5, Fig. 6).

The soft tissues were adapted to permit healing by first intention.

The suturing technique was characterized by the mobilization of a muco-periostal flap. In all of the cases, resorbable material (Vycril® 4/0) was used for suturing. Simple detached stitching was performed to obtain a hermetic closure at the wound margins. Complete primary closure of mucosa protects the area from infection and reduces pain.

Written instructions for proper oral hygiene for maintenance of the surgical site were given to all patients.

Programmed monitoring of mucosal healing was carried out postoperatively at 3, 7, and 14 days (when the sutures were removed) and at 21, 30, 60, and 90 days (Fig. 7). Radiographic evaluation was carried out at six months, one year and every year after the first by an orthopantomography, a CT scan and clinical evaluation (Fig. 8). The study group had a total follow-up period ranging from 48 to 50 months.

The patients were always examined by detecting the clinical signs of BRONJ that were mentioned above: pain, swelling, non-healing, exposed necrotic bone, and/or fistulas with connection to the bone. The absence of both clinical and radiographic signs was determined to indicate successful treatment.24
Discussion

BPs are drugs used to prevent and treat secondary skeletal-related events (SREs) that are due to bone metastasis from solid cancer and multiple myeloma. The goal of treatment with BPs is to improve patients’ quality of life. BRONJ is a term that has recently emerged to describe an important complication in some patients receiving this class of drugs. It is difficult to precisely establish the incidence of BRONJ in patients treated with i.v. BPs. According to AAOMS studies, the annual incidence ranges from 0.8% to 12%. Among BPs, zoledronic acid is the chemical compound frequently associated with BRONJ, due to its major pharmacological efficiency and its wider use than the other BP.

Moreover, the duration of BPs administration is important. In fact, the risk of developing BRONJ is directly proportional to the duration of the therapy.

It has been established that poor oral hygiene is a strong risk factor for this illness, in addition to poor oral hygiene and periodontal disease. Also, in most cases, BRONJ is associated with an oral surgery procedure, usually dental extraction. According to the literature, tooth extraction is the cause of BRONJ in 40–86% of cases. Other possible causes of BRONJ are dental implants, periodontal abscess or disease, and extraction of impacted teeth. However, necrosis can occur spontaneously in some cases. Nevertheless, it is presumable that when an initiating cause is not evident, chronic or latent infections and traumas can be the cause of BRONJ.

It is known that BPs inhibit osteoclastic activity, and they also reduce normal bone turnover, which may result in osteonecrosis of the traumatized bone. Moreover, BPs show anti-angiogenetic activity: therefore, the bones of patients treated with BPs are poorly vascularized and poorly supplied with substances necessary for wound healing, such as oxygen, growth factors and other mediators. Furthermore, bacterial infections in the mouth and residual teeth reach the necrotic bone. The reduced macrophagic activity that is induced by BPs decreases the immune response of the patient even further.

BPs soft tissue toxicity is another complication associated with these drugs that can play a role in BRONJ etiology. In fact, BPs are toxic on gastric mucosal cells and intestinal epithelial cells. A recent, interesting case report described the direct toxicity of alendronate on the palatal mucosa. A patient developed a palatal ulceration after holding alendronate tablets under a removable denture; this suggests a potentially toxic effect of BPs on oral mucosa similar to that seen in gastrointestinal studies. The mucosal damage can then provide access to the underlying bone for oral bacteria, which would lead to bone infection and necrosis.

In a recent study, Landesberg et al. proposed that oral epithelial cells are subjected to local increases in BP concentration after a traumatic event, and the presence of such BPs may inhibit normal epithelial wound healing. This would contribute to the persistent exposure of underlying bone and the development of ONJ. The study demonstrated that BP pretreatment of oral mucosal cells inhibits proliferation and wound healing at clinically relevant doses, and this inhibition is not due to cellular apoptosis.

The described scenario explains why bisphosphonate-related necrosis is localized only in the jaw, even though these drugs are systemically administered and the entire skeletal system absorbs the drugs in the same way. There are no other bones in the organism that directly participate in such a strongly septic environment as the oral cavity. Furthermore, continuous mechanical stimulations of the maxillary produce a high turnover of cells, accounting for major BP uptake.

Even though primary prevention of BRONJ has been demonstrated to be effective in reducing the onset of new cases of osteonecrosis, the high frequency of BP prescriptions and the unpredictable...
onset of BRONJ will result in an increasing number of BRONJ cases. Several therapies have been proposed for BRONJ therapy, including conservative topical treatment, conservative surgical treatment or surgical resection, and hyperbaric or ozone therapy. However, none of these produced effective results in most cases.

Some researchers have proposed and successfully used platelet rich plasma (PRP) for the treatment of BRONJ. PRP was first introduced by Marx, in combination with autologous bone grafts, for the reconstruction of mandibular defects. Few BRONJ patients reported in the literature have been treated with PRP (collectively, 17 patients). Moreover, PRP has often been associated with other therapies, such as hyperbaric therapy and resorbable membranes, confounding the outcome of the treatment.

PRP therapies have been proposed as complements to conservative surgery in cases that do not recover with conservative topical treatments. Adornato and colleagues treated twelve patients who presented with soft tissue ulcerations and bone exposure with measurements ranging from 5 to 25 mm. These lesions had not responded to six months of treatment with cleaning therapies, 0.12% chlorhexidine rinses and intermittent antibiotic therapies. These patients were treated with conservative marginal resections of alveolar bone with primary closure over the bony defect, PRP and a resorbable membrane under antibiotic coverage. After six months, ten patients showed complete soft tissue healing, and one patient showed a recurrence of epithelial dehiscence. One patient, with recovery by secondary intention, did not show any regression of the bone exposure.

The rational base for the successful employment of PRP in patients affected by BRONJ rests on the assumption that the presence of growth factors, usually inhibited by BPs, represents a substitute for bone healing that is similar to the physiological one. As mentioned above, BPs present anti-angiogenic activity in bone. This effect is due to the reduction of the levels of vascular endothelial growth factor (VEGF), which is noticed up to 21 days after infusion.

In 1999, Anitua proposed the use of plasma rich in growth factors (PRGF) in dentistry. PRGF offers several advantages, including the simultaneous release of many growth factors (platelet-derived growth factor, transforming growth factor-β, endothelial growth factor, vascular endothelial growth factor, insulin-like growth factor-1, basic fibroblast growth factor, and hepatocyte growth factor), and it is an autologous product. VEGF and PDGF are potent mitogen factors: the first one acts on endothelial cells, while the second stimulates the proliferation of osteoblasts and influences bone reabsorption mediated by osteoclasts. It has been suggested that bisphosphonates, by reducing VEGF and PDGF levels, can have anti-tumor activity. Particularly, zoledronic acid seems to be able to reduce levels of platelet-derived growth factor (PDGF). Consequently, the biological properties of growth factors in PRGF suggest its local application for BRONJ. Theoretically, the angiogenetic ability of PRGF can promote rapid formation of the blood supply to bone and enhance cell migration in patients affected by BRONJ. This can contribute to rapid remodeling. As confirmed by Lopez-Jornet et al., growth factors that are applied to wounds can accelerate healing by stimulating angiogenesis, tissue maturation and epithelialization. In a study on rabbits, Lopez-Jornet et al. suggested that the application of PRGF accelerates epithelialization and reduces inflammation at 28 days for provoking wounds in the tongue, which is a moist area where saliva and maceration of the tissue during mastication may initially interfere with the healing process. Our results confirm this study. Contrary to Carlson and Basile, who obtained 92% success without the use of a preparations rich in growth factors, we have obtained 100% success. PRGF allowed us to obtain a complete primary closure of the mucosa, which protects the area from infection and reduces sintomatology.

All of the benefits described above indicate that the use of preparations rich in growth factors (PRGF) may offer support for BRONJ surgical treatment, especially in cases where there is a need to stimulate the healing process.

Conclusion

In conclusion, our data on the use of a preparation rich in growth factors indicate positive results regarding the surgical treatment of BRONJ, even if the analyzed sample is small. However, it is important to note that the positive results have a total follow-up period ranging from 48 to 50 months. The long follow-up period represents a good indication in continuing this kind of analysis. Therefore, we suggest that surgical treatment on patients taking BPs is necessary because it improves and accelerates the healing process. PRGF may accelerate epithelial wound healing, reduce tissue inflammation after surgery, enhance regeneration of osseous and epithelial tissues and promote tissue vascularization.

Conflict of interest statement

None declared.

References


