A Report on a 7-year Follow up of the Surgical Management with PRGF®-ENDORET® of Oncologic Patients Affected by Intravenous Bisphosphonate Related Osteonecrosis of the Jaw

Marco Mozzati¹, Giorgia Gallezio^{1*}, Renato Pol², Giuliana Muzio³, Rosangela Canuto⁴ and Laura Bergamasco⁵

¹SIOM Oral Surgery and Implantology Center, Turin, Italy

²Consultant, Oral Surgery Unit, Dentistry Section, Department of Clinical Physiopathology, University of Turin, Italy

³Department of Experimental Medicine and Oncology, University of Turin, Turin, Italy

⁴Full Professor Department of Experimental Medicine and Oncology, University of Turin, Turin, Italy

⁵Full Professor Department of Surgical Sciences, University of Turin, Turin, Italy

Abstract

BRONJ is an important complication in bisphosphonate therapy that dramatically influences the patient's quality of life and requires immediate intervention. The situation is worsened by the fact that its management is still an open issue, with no definitive standard of care. The aim of this paper is to present the short, middle and long term (7 years) results of surgical treatment of 32 BRONJ cases involving the use of PRGF®-ENDORET®. No intraoperative complications were observed; the short period freedom from light complications was 84.4%, with complete remission in a few weeks; after 7 years the freedom from complications and need of re intervention is 100%. The freedom from onset of a new BRONJ on untreated sites was 100% up to 4 years after which decreased to 82%. The surgical procedure with the applications of platelet-enriched preparations can thus be considered favorably tested, having led to rapid osseous remodeling and to a satisfactory closure of the mucosa thus shielding the area from infection and reducing symptomatology.

Keywords: BRONJ; Bisphosphonates; Platelet-enriched preparations; Mucosa healing; Bone healing; PRGF-ENDORET

Introduction

Intravenous bisphosphonates (BPs) are the standard therapy in the management of patients with metabolic imbalance involving high bone turnover and increased bone restoration, such as malignant hypocalcaemia, bone metastasis associated with solid tumours and multiple myeloma. In the past years its potentially negative side effects have however caused growing concern; in particular the profound bone remodeling inhibition can cause Bisphosphonate Related Osteonecrosis of the Jaw (BRONJ), defined as an avascular area of necrotic bone in the maxillofacial area, with or without exposed bone, unable to heal within 8 weeks after its identification by the health care provider [1] in a patient who is or was receiving BP therapy and does not have a previous history of irradiation in the maxillofacial region. The onset of BRONJ is not always easy to detect, and this may favor a progressive involvement of a large part of the maxillary and mandibular bone with increasing complications [2].

At the present time, the pathophysiological mechanisms underlying BRONJ are not yet elucidated, even if its spontaneous occurrence suggests a multifactorial pathogenesis. One hypothesis is that BPs dampens maxillary and mandibular bone turnover, reducing osteoblast proliferation and osteogenic properties and increasing the ability of the mucosa cells to induce osteoclast differentiation and inflammatory processes. On the contrary, in other bones, BPs shows an inhibitory effect on osteoclast cell function. A recent study reported that zoledronic acid, released from the bone, affects oral mucosa, inducing early apoptosis and subsequently reducing cell growth [3]. In 2012 a study *in vitro* evidenced that when epithelial cells are exposed to zoledronic acid, the latter can affect the properties of epithelial cells themselves, of osteoblasts and osteoclasts, all of which contribute to the onset of BRONJ [4]. These results have been confirmed in a subsequent study *in vivo* [5]. Moreover, BPs also possesses anti-angiogenic effect, which probably compromise post-extraction healing [5,6].

Since the exact BRONJ pathogenesis has not yet been established and seems to involve several mechanisms, including both hard and

soft tissue damage, no agreement on the most appropriate BRONJ management and no definitive standard of care have been reached [7-11]. Active search for surgical protocols that may favor both bone and mucosal healing processes, while concurrently limiting surgical damage, must go on to respond to the needs of patients who are treated with BPs.

In a previous paper we reported on a trial involving 32 patients affected by BRONJ who were treated surgically using an autologous platelet-enriched preparation. The results were satisfactory, proving on the short and middle term the effectiveness of such preparations as a supplementary source of stimulation to the physiological deficit, able to promote angiogenesis as well as bone and mucosal wound healing [12-14].

The aim of the present paper is to evaluate the PRGF®-ENDORET® effectiveness on the surgical treatment of BRONJ also on the long term, through a clinical and radiographic follow-up up to 7 years after surgery.

Materials and Methods

In a previous paper [15] we described the surgical protocol followed in the management of 32 oncologic patients affected by intravenous bisphosphonate related osteonecrosis of the jaw with lesions meeting Marx IIB classification and who had no other kind of treatment before surgical procedures [16].

***Corresponding author:** Giorgia Gallezio, SIOM Oral Surgery and Implantology Center, Corso Dante 64, 10126 Turin, Italy, E-mail: giorgiagallezio@libero.it

Received March 07, 2013; **Accepted** April 20, 2013; **Published** May 05, 2013

Citation: Mozzati M, Gallezio G, Pol R, Muzio G, Canuto R, et al. (2013) A Report on a 7-year Follow up of the Surgical Management with PRGF®-ENDORET® of Oncologic Patients Affected by Intravenous Bisphosphonate Related Osteonecrosis of the Jaw. Surgery S12: 011. doi:10.4172/2161-1076.S12-011

Copyright: © 2013 Mozzati M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Using the new classification of Bedogni et al. [17], all 32 lesions were at stage 2: among them, 12 were asymptomatic (Stage 2a) and 20 symptomatic (Stage 2b). In all cases, BRONJ was clinically diagnosed and confirmed radiographically by TC [18].

The possible causes of the diagnosed BRONJ were dental surgery, periodontal diseases and ill-fitting dentures. Subjects with recurrent BRONJ onsets and osteoporotic patients assuming zoledronic acids were excluded. Operations (24 in the mandible and 8 in the maxilla) were performed in 2006 at the Oral Surgery Department of the Dental School of the University of Torino, Italy. Dental extraction was the suspected cause of BRONJ in 17 cases. After the first period of close observation, from June 2006 to October 2010, the follow-up visits were scheduled every 6 months, up to December 2012. The patient baseline is detailed in tables 1 and 2.

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

The surgical protocol was described in details in our previous article [15]; in short, it consisted in root scaling and oral hygiene instructions one week before surgery. All patients were administered antibiotics amoxicillin and clavulanic acid at a dosage of 1 tablet every 8 hours for a total of 10 days, starting from the evening before the surgical appointment.

The PRGF was obtained following the protocol described by Anitua [12]. Before anesthesia, 10-20 mL of blood was drawn from the peripheral vein of each patient using 5 mL tubes containing 3.8% trisodium citrate solution as an anticoagulant. The tubes were centrifuged at 1800 rpm for 8 min (PRGF®-ENDORET® System, BTI Biotechnology Institute, Milan, Italy) at room temperature to enhance separation of blood into its three basic components: red blood cells (at the bottom of the tube), PRGF (in the middle), and plasma poor in growth factors (PPGF) at the top. The PRGF was collected from each tube and transferred in sterile tubes with addition of Calcium chloride (50 IL) at 10% for each 1 mL fraction of PRGF. The formation of the PRGF gel took about 15 - 20 min, after which it was ready to be used for filling the bone defect [19].

Local anesthesia was done with 2% mepivacaine to reduce restriction of the blood supply. Surgery consisted in resecting all parts of BRONJ [20,21]. Resection margins were pre-surgically evaluated radio graphically by TC and determined by the clinical appearance of bleeding bone. 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 improve healing and prevent bleeding. The suture was characterized by the mobilization of a muco-periosteal flap to permit healing by first intention. Simple detached stitching with Vycril 4/0 was performed to get a hermetic closure at the wound margins. Written instructions for postoperative course and oral hygiene aimed at a good maintenance of the surgical site were given to all patients.

Clinical controls were performed postoperatively at 3, 7, 14, 21, 30, 60, and 90 days. Radiographic evaluations were carried out at six months, and then every year by an orthopantomography, a TC and clinical evaluation. The patients were always examined for possible clinical signs of BRONJ: pain, swelling, non-healing, exposed necrotic bone and/or fistulas with connection to the bone [22].

Statistical analysis: the freedom from adverse events was computed for the short period (a few weeks) as a simple percentage with 95%

Confidence Interval; for the long term (seven years) we followed the standard procedure of computing the Kaplan Meyer probability curve which takes into account the actual period of observation for each patient.

Results

No patients had to interrupt the use of i.v BPs because of surgery need. The oncologist, when needed, determined drug holiday. After surgery, five patients had some minor discomforts with complete remittal of all complications in a few weeks: resolution of disease was defined as the maintenance of mucosal closure without clinical and radiographic signs of residual infection or exposed bone at the time of evaluation. The short time success rate of our surgery thus amounts to 84.4% (95%confidence interval 68.7-94.0%).

During the follow up period (2006-2012) 7 patients died for oncologic reasons. During these seven years no complication was reported and no surgical re-intervention on the treated site was needed for any of the patients. We can thus claim a satisfactory 100% freedom from adverse events as well as from re-intervention.

5 patients, 3 women and 2 men all with multiple myeloma, after being surgically treated with success, developed a new BRONJ in another site in the oral cavity. The new BRONJ were 4 in mandible and 1 in maxilla. Clinical signs were pain, swelling, no healing, exposed

		Study group (number)
Gender	Males	10
	Females	22
Age (yrs)	44-60	7
	60-70	16
	70-83	9
Smoking habit	No	20
	<15/day	9
	>15/day	3
Other medications	Steroids	11
	Chemotherapy	4
Primary disease	Prostatic carcinoma	6
	Breast carcinoma	5
	Multiple myeloma	14
	Lung carcinoma	4
	Ovarian carcinoma	3
Drug prescribed	Zoledronic acid	26
	Pamidronate	6

Table 1: Some patients characteristics at the time of the diagnosis (Mozzati et al. 2012).

		Study group number
Location of BRONJ	Mandible	24
	Maxilla	8
Cause for BRONJ	Tooth extraction	17
	Ill-fitting dentures	7
	Periodontal disease	8
Pain	+	20
	-	12
Presence of pus	+	15
	-	17
Exposed/necrotic bone	+	21
	-	11
Oral fistulas	+	15
	-	17

Table 2: Some BRONJ characteristics at the time of the diagnosis (Mozzati et al. 2012).

necrotic bone and/or fistulas with connection to the bone. Also BRONJ was diagnosed and confirmed radio graphically with TC.

The Kaplan-Meier curve of freedom from a new BRONJ on untreated sites for patients who underwent a previous BRONJ surgery is shown in figure 1; it is 100% up to 4 years and afterwards decreases to 82% (95% CI 70-92%). Since the departure from 100% freedom falls around 4.5-5 years, we obtain an indication about the time at which patients should be most closely monitored for the arousal of possible new problems. All 5 second BRONJ patients were successfully managed with our surgical protocol using PRGF-ENDORET®, without any surgery complications or evidence for postoperative bisphosphonate-associated osteonecrosis of the jaw at 8 months follow up.

Discussion and Conclusion

In recent years many therapies have been suggested for BRONJ treatment, including conservative topical treatment, conservative surgical treatment or surgical resection, and hyperbaric or ozone therapy [23-27]. In 2007, Lee et al. have proposed and successfully managed 2 BRONJ using platelet- enriched preparations like platelet rich plasma (PRP) [28].

In our surgical trial on 32 BRONJ patients, we proposed the use of Platelet Rich in Growth Factors (PRGF-ENDORET®) [15]. The goal was to exploit their angiogenetic ability to promote rapid formation of the blood supply to the bone and enhance cell migration in patients affected by BRONJ, counterbalancing the antiangiogenic action which induces reduced capillary formation and inhibition of endothelial and vascular growth factors thus leading to avascular necrosis. This effect of BPs was also demonstrated by authors with “in vitro” experiments, animal models, and human studies on BP-treated patients with advanced solid cancer and bone metastasis [29].

The rational base for the employment of platelet-enriched preparations in patients affected by BRONJ rests on the assumption that the presence of growth factors, usually inhibited by BPs, represents substitute stimulation for a bone healing process similar to the physiological one. Particularly BPs seem to inhibit the action of vascular endothelial growth factor (VEGF) and the formation of new capillaries, other than to inhibit hydroxymethylglutaryl coenzyme A reductase (HMGR) involved in osteoblast proliferation [4,5]. The application of PRGF-ENDORET® provides hundreds of proteins and growth factors to the local milieu including angiogenic factors (VEGF and angiopoietin), and factors that promote osteogenic differentiation, which can activate and accelerate the regeneration of the involved tissues

[30]. In fact, PRGF increases TGFβ, a growth factor involved in the stimulation of osteoblast proliferation and differentiation, extracellular matrix production, and VEGF expression. Moreover, PRGF increases BMP-7, a growth factor involved in osteogenesis [19]. PRGF can contribute to a rapid osseous remodeling and to a complete primary closure of the mucosa that protects the area from infection and reduces symptomatology.

The results of our trial are to be considered satisfactory: the freedom from light complications immediately after surgery was 84.4%, whereas the freedom from adverse events and re-intervention in the 7-year follow-up period was 100%. The Kaplan-Meier actuarial curve for freedom from a new BRONJ on untreated sites was 100% up to 4 years and afterwards decreased to 82%, where it remained constant up to 7 years.

It is important to highlight that none of the present patients interrupted bisphosphonate therapy. The treating specialist, considering whether discontinuation of the drugs could increase the risk of skeletal complications, achieved this decision. This therapeutic choice did not interfere with the success of the surgery. Our results are similar to those obtained from Curi et al. in another study [30,31]: there is no reason to interrupt bisphosphonate therapy when surgical treatment is indicated.

Finally, this is one of the few studies describing a surgery protocol for BRONJ with very good results on a many years follow-up [27].

References

- Khosla S, Burr D, Cauley J, Dempster DW, Ebeling PR, et al. (2007) Bisphosphonate-associated osteonecrosis of the jaw: report of a task force of the American Society for Bone and Mineral Research. *J Bone Miner Res* 22: 1479-1491.
- Ruggiero SL (2009) Bisphosphonate-related osteonecrosis of the jaw (BRONJ): initial discovery and subsequent development. *J Oral Maxillofac Surg* 67: 13-18.
- Allen MR, Burr DB (2008) Mandible matrix necrosis in beagle dogs after 3 years of daily oral bisphosphonate treatment. *J Oral Maxillofac Surg* 66: 987-994.
- Scheper M, Chaisuparat R, Cullen K, Meiller T (2010) A novel soft-tissue in vitro model for bisphosphonate-associated osteonecrosis. *Fibrogenesis Tissue Repair* 3: 6.
- Saracino S, Canuto RA, Maggiora M, Oraldi M, Scoletta M, et al. (2012) Exposing human epithelial cells to zoledronic acid can mediate osteonecrosis of jaw: an in vitro model. *J Oral Pathol Med* 41: 788-792.
- Mozzati M, Martinasso G, Maggiora M, Scoletta M, Zambelli M, et al. (2013) Oral mucosa produces cytokines and factors influencing osteoclast activity and endothelial cell proliferation, in patients with osteonecrosis of jaw after treatment with zoledronic acid. *Clin Oral Investig* 17: 1259-1266.
- Carlson ER, Basile JD (2009) The role of surgical resection in the management of bisphosphonate-related osteonecrosis of the jaws. *J Oral Maxillofac Surg* 67: 85-95.
- Pautke C, Bauer F, Tischer T, Kreutzer K, Weitz J, et al (2009). Fluorescence-guided bone resection in bisphosphonate-associated osteonecrosis of the jaws. *J Oral Maxillofac Surg* 67:471-476.
- Scoletta M, Arduino PG, Dalmaso P, Broccoletti R, Mozzati M (2010) Treatment outcomes in patients with bisphosphonate-related osteonecrosis of the jaws: a prospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 110: 46-53.
- Scoletta M, Arduino PG, Reggio L, Dalmaso P, Mozzati M (2010) Effect of low-level laser irradiation on bisphosphonate-induced osteonecrosis of the jaws: preliminary results of a prospective study. *Photomed Laser Surg* 28: 179-184.
- Stockmann P, Vairaktaris E, Wehrhan F, Seiss M, Schwarz S, et al. (2010) Osteotomy and primary wound closure in bisphosphonate-associated osteonecrosis of the jaw: a prospective clinical study with 12 months follow-up. *Support Care Cancer* 18: 449-460.

12. Anitua E, Sánchez M, Orive G, Andía I (2007) The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. *Biomaterials* 28: 4551-4560.
13. Molina-Miñano F, López-Jornet P, Camacho-Alonso F, Vicente-Ortega V (2009) Plasma rich in growth factors and bone formation: a radiological and histomorphometric study in New Zealand rabbits. *Braz Oral Res* 23: 275-280.
14. Anitua E, Orive G, Pla R, Roman P, Serrano V, et al. (2009) The effects of PRGF on bone regeneration and on titanium implant osseointegration in goats: a histologic and histomorphometric study. *J Biomed Mater Res A* 91: 158-165.
15. Mozzati M, Galesio G, Arata V, Pol R, Scoletta M (2012) Platelet-rich therapies in the treatment of intravenous bisphosphonate-related osteonecrosis of the jaw: a report of 32 cases. *Oral Oncol* 48: 469-474.
16. Marx R. (2007) Oral and intravenous bisphosphonate-induced osteonecrosis of the jaws: history, etiology, prevention, and treatment. Hanover Park, I.L.: Quintessence Publishing Co., Inc
17. Bedogni A, Fusco V, Agrillo A, Campisi G (2012) Learning from experience. Proposal of a refined definition and staging system for bisphosphonate-related osteonecrosis of the jaw (BRONJ). *Oral Dis* 18: 621-623.
18. Bianchi SD, Scoletta M, Cassione FB, Migliaretti G, Mozzati M (2007) Computerized tomographic findings in bisphosphonate-associated osteonecrosis of the jaw in patients with cancer. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 104: 249-258.
19. Mozzati M, Martinasso G, Pol R, Polastri C, Cristiano A, et al. (2010) The impact of plasma rich in growth factors on clinical and biological factors involved in healing processes after third molar extraction. *J Biomed Mater Res A* 95: 741-746.
20. Mozzati M, Arata V, Galesio G, Carossa S (2011) A dental extraction protocol with plasma rich in growth factors (PRGF) in patients on intravenous bisphosphonate therapy: a case-control study. *Joint Bone Spine* 78: 648-649.
21. Mozzati M, Arata V, Galesio G (2012) Tooth extraction in patients on zoledronic acid therapy. *Oral Oncol* 48: 817-821.
22. Wilde F, Heufelder M, Winter K, Hendricks J, Frerich B, et al. (2011) The role of surgical therapy in the management of intravenous bisphosphonates-related osteonecrosis of the jaw. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 111: 153-163.
23. Magopoulos C, Karakinaris G, Telioudis Z, Vahtsevanos K, Dimitrakopoulos I, et al. (2007) Osteonecrosis of the jaws due to bisphosphonate use. A review of 60 cases and treatment proposals. *Am J Otolaryngol* 28: 158-163.
24. Montebugnoli L, Felicetti L, Gissi DB, Pizzigallo A, Pelliccioni GA, et al. (2007) Bisphosphonate-associated osteonecrosis can be controlled by nonsurgical management. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 104: 473-477.
25. Wutzi A, Biedermann E, Wanschitz F, Seemann R, Klug C, et al. (2008) Treatment results of bisphosphonate-related osteonecrosis of the jaws. *Head Neck* 30: 1224-1230.
26. Agrillo A, Filiaci F, Ramieri V, Riccardi E, Quarato D, et al. (2012) Bisphosphonate-related osteonecrosis of the jaw (BRONJ): 5 year experience in the treatment of 131 cases with ozone therapy. *Eur Rev Med Pharmacol Sci* 16: 1741-1747.
27. Vescovi P (2012) Bisphosphonates and osteonecrosis: an open matter. *Clin Cases Miner Bone Metab* 9: 142-144.
28. Lee CY, David T, Nishime M (2007) Use of platelet-rich plasma in the management of oral bisphosphonate-associated osteonecrosis of the jaw: a report of 2 cases. *J Oral Implantol* 33: 371-382.
29. Landesberg R, Woo V, Cremers S, Cozin M, Marolt D, et al. (2011) Potential pathophysiological mechanisms in osteonecrosis of the jaw. *Ann N Y Acad Sci* 1218: 62-79.
30. Anitua E, Begoña L, Orive G (2012) Treatment of hemimandibular paresthesia in a patient with bisphosphonate-related osteonecrosis of the jaw (BRONJ) by combining surgical resection and PRGF-Endoret. *Br J Oral Maxillofac Surg*.
31. Curi MM, Cossolin GS, Koga DH, Zardetto C, Christianini S, et al. (2011) Bisphosphonate-related osteonecrosis of the jaws--an initial case series report of treatment combining partial bone resection and autologous platelet-rich plasma. *J Oral Maxillofac Surg* 69: 2465-2472.