Bisphosphonates (BP) are nonhormonal medications used in the treatment of various bone malignancies and metabolic conditions. Since 2003, there have appeared a significant and growing number of articles in the worldwide medical and dental literature describing the complication of an osteonecrosis of the jaws associated with the intravenous and, most recently, the oral form of BP medication that has been refractory to any definitive form of treatment. The authors have successfully managed 2 patients taking the oral form of BP with adjunctive treatment using platelet-rich plasma (PRP), and in one case with hyperbaric oxygen (HBO). We were able to obtain complete remission in each case, which is defined as resolution of pain and complete closure of exposed bone in the jaws. The purpose of this report is to describe a treatment protocol and the rationale for using PRP and HBO to obtain complete remission of this new pathologic condition.

Key Words: osteonecrosis; platelet-rich plasma; angiogenesis; neovascularization; bisphosphonates

INTRODUCTION

Bisphosphonate (BP) drugs are synthetic analogs of inorganic pyrophosphate and have an affinity for calcium. Intravenous and oral BPs are highly prescribed pharmacologic agents used to manage a variety of malignant and benign metabolic conditions, such as Paget's disease of bone; multiple myeloma; hypercalcemia of malignancy; metastatic lesions involving the thyroid gland, prostate gland, and breast carcinoma; osteoporosis; osteopenia; fibrous dysplasia; and, recently, osteogenesis imperfecta in pediatric patients. Between 2001 and 2004, the oral BP alendronate (Fosamax, Merck, Whitehouse Station, NJ), was the most commonly prescribed nonhormonal drug used to treat osteoporosis. The National Osteoporosis Foundation has estimated that 10 million people are diagnosed with osteoporosis and another 44 million people are at risk for developing this disease. It is estimated that over 90% of drug side effects are not reported to the U.S. Food and Drug Administration. Common side effects reported with use of BP drugs include esophageal irritation, nausea and vomiting, and bone and muscle discomfort. Recently, it was reported that BP drugs may also cause an avascular necrosis of alveolar bone in both the maxilla and mandible that has no definitive cure and can occur spontaneously or after an oral surgical procedure. The
osteonecrosis of the jaws is attributable to inadequate tissue perfusion.\textsuperscript{5–7} Since the first report of this new pathologic entity, numerous terms have been applied describing this clinical condition, such as BP-associated osteonecrosis,\textsuperscript{8,9} osteonecrosis of the jaw (ONJ),\textsuperscript{10} osteochemonecrosis (bis-phossy jaw),\textsuperscript{11} and, most recently, BP-related osteonecrosis of the jaw.\textsuperscript{12} The purpose of this report is to describe our adjunctive treatment protocol and the rationale for using platelet-rich plasma (PRP) and hyperbaric oxygen (HBO) to obtain complete remission of this new pathologic entity in 2 patients who were prescribed the oral form of BPs by their physician to manage the skeletal effects of osteoporosis.

**Review of the Literature**

In 2003, in a letter to the editor published in the *Journal of Oral and Maxillofacial Surgery*, Marx\textsuperscript{5} reported 36 cases of necrotic bone infections that resembled osteoradionecrosis (ORN) after patients had undergone surgery of the jaws, mostly after extraction of teeth. The osseous destruction was described as an avascular necrosis possibly attributable to intravenous BP use. That same year, Carter and Gross\textsuperscript{6} reported 5 additional cases of osteonecrosis of the jaws. In 2004, Ruggiero et al\textsuperscript{13} described 63 cases of avascular necrosis of the jaws with similar clinical findings as reported by Marx.\textsuperscript{5} In 2005, Hellstein and Marek\textsuperscript{11} reported 28 cases of osteochemonecrosis, which they coined bis-phossy jaw. In all of the cases documented by the authors, patients presented with many of the following signs and symptoms: nonhealing extraction sites, bone pain, exposed bone with or without pain, soft-tissue swelling, purulent exudate, inflammation, and orocranial fistulas.\textsuperscript{5–13} However, some patients with ONJ were asymptomatic.\textsuperscript{14} In these patients, the only clinical finding was exposed bone of the maxilla or mandible. More recently, additional cases have been reported, but in patients taking the oral form of these drugs.\textsuperscript{12} Since these reports, ONJ from BP use is now recognized as a new clinical entity in oral and maxillofacial surgery and pathology.

At present, there is no definite causal relationship between ONJ and BP therapy.\textsuperscript{5–10} More importantly, no definitive treatment protocol is currently available that has been shown to be effective in treating ONJ.\textsuperscript{5–14} But, a correlation does exist with BP use and ONJ based on the reporting of our colleagues.\textsuperscript{5–14} Therefore, other risk factors or comorbidities should be considered in this group of patients. Based on the limited experience and knowledge from oncology patients, possible comorbidities for ONJ may include a compromised immune system from the malignancy, radiation to the head and maxillofacial structures, chemotherapy, diabetes, cardiovascular disease, and use of corticosteroids and estrogen. Additional comorbidities may include advanced age, use of tobacco or alcohol, existing periodontal disease, dental decay with abscess, and endodontic failure.\textsuperscript{6,14–17}

**Mechanism of Pathophysiology**

The exact mechanism of how exposed bone in the jaw occurs is not known.\textsuperscript{5,11,15,17} The most accepted theory is that exposed bone is the result of a disruption of the osteoblast-osteoclast homeostatic cycle due to BPs.\textsuperscript{5,15} This homeostatic cycle was also referred to as the osteoclast-osteoblast axis by Hellstein and Marek.\textsuperscript{11} Alteration of this axis may be the most important risk factor for developing ONJ.

The BPs have an affinity for binding to the mineral matrix of bone and their primary pharmacologic effect is the inhibition of bone resorption by decreasing osteoclast function.\textsuperscript{15,18–20} Because of this specific pharmacologic effect, the number of osteoclasts will be increased in osteolytic lesions. With increased osteoclastic activity, cellular activity of bone remodeling and resorption is disrupted. The BPs will prevent differentiation into osteoclasts by monocytes and macrophages and will stimulate apoptosis of osteoclasts. With disruption of the osteoblast-osteoclast homeostatic cycle, osteoclast activity remains unaffected, which results in increased bone mass and density.\textsuperscript{11–15} The BP drugs are not metabolized and can remain in bone for many years impairing the homeostatic cycle of bone remodeling and repair.\textsuperscript{11,15} The BPs have also been shown to affect vascularization and inhibit angiogenesis. In a rat model, Fournier et al\textsuperscript{21} demonstrated that BP agents were able to inhibit angiogenesis and vascular endothelial growth factor (VEGF). In another study demonstrating the effects of BP drugs on the vasculature, Wood et al\textsuperscript{22} showed that BPs inhibit vascular endothelial cell proliferation. Therefore, an alternative mechanism leading to ONJ could be responsible for the antiangiogenic effects of BPs.

**Current Recommendations for Established ONJ**

The clinical diagnosis of ONJ is based on the patient’s history, physical examination, and use of various imaging studies such as radiographs and computerized tomography (CT). Currently, there is no consensus definition of ONJ. A clinical diagnosis is confirmed
when there has been no evidence of healing after 6 weeks and no evidence of metastatic disease in the jaw.\textsuperscript{12}

Because of the paucity of long-term data on treatment outcomes, there is no accepted treatment protocol to completely eliminate this necrosis of bone, as the pathogenesis is not entirely clear.\textsuperscript{5,11,15} The goal of treatment is to eliminate pain and to control the progression of infection and bone necrosis.\textsuperscript{8,10,12,15,17} Although HBO therapy has been used in the management of ONJ, it was determined to be ineffective.\textsuperscript{8,10,13,15,23}

In documented cases, aggressive surgical debridement is not recommended, as it can worsen the condition by causing further exposure of bone.\textsuperscript{7–10,14,15,17} Use of soft tissue flaps to cover areas of exposed bone is also not recommended, because of possible dehiscence of the flaps, which results in more bone exposure. Therefore, minimally invasive procedures have been suggested, such as surface debridement to remove bone sequestra and smoothing of sharp bone spicules to reduce trauma to the soft tissues overlying the gingival and mucosa. Exposed surfaces of the maxilla or mandible have proven difficult to treat and can be left exposed.

Use of penicillin-type antibiotics has been shown to be of benefit.\textsuperscript{12,14,17} Use of oral mouth rinses with 0.12% chlorhexidine is also recommended as it may prevent progression to ONJ.\textsuperscript{12,14,17} Biopsy of the exposed bone is not recommended, unless malignancy or metastasis must be ruled out.\textsuperscript{12} Cultures for microbiotic flora to identify pathogens and to determine appropriate antibiotic therapy can also be obtained at the time of the biopsy procedure.

**Rationale for PRP therapy**

To the best of our knowledge, there are no reports in the world literature describing the use of PRP in the management of ONJ of the jaws or avascular necrosis caused by other mechanisms. Use of PRP has been shown to be of benefit in soft and hard tissue healing.\textsuperscript{24,25} PRP contains 4 to 6 times the normal level of growth factors compared with peripheral platelet counts.\textsuperscript{24} Normal human platelet counts are in the blood range of 150 000/\(\mu\)L to 350,000/\(\mu\)L. The average platelet count is approximately 200 000/\(\mu\)L. Specifically defined, PRP is a platelet concentration with at least 1 000 000/\(\mu\)L in a 5-mL volume of plasma.\textsuperscript{24,26}

Besides the procoagulant effects of platelets, PRP is a source of growth factors involved in wound healing via clot formation.\textsuperscript{24} In the early stages of wound healing, such as bone fractures and surgery, platelets are activated by the coagulation cascade. The activated platelets will release the contents of the alpha secretory granules into the site of wound injury.\textsuperscript{24–28} Adding thrombin and calcium chloride to PRP has also been shown to activate the alpha granules to release the following active, biologic growth factors: platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-b), VEGF, insulin-like growth factor I, epidermal growth factor (EGF), epithelial cell growth factor, and TGF-b1 and TGF-b2.\textsuperscript{24,26} These growth factors will recruit undifferentiated mesenchymal stem cells to the site of injury and stimulate mitosis of these cells. This will, in turn, stimulate mesenchymal stem cells to differentiate into osteoblasts at the site of tissue injury.\textsuperscript{25,29}

One of the primary growth factors produced by platelets, macrophages, and endothelial cells is PDGF, which has been identified as an important protein for hard- and soft-tissue healing.\textsuperscript{24,29} But platelets contain the greatest source of this specific growth factor, which stimulates wound healing by promoting granulation tissue formation, mitosis, vascular formation, macrophage activation, and activation of other growth factors.\textsuperscript{24,29,30} At the site of a wound, PDGF has been shown to stimulate chemotaxis (recruitment) and mitogenesis (migration) of stem cells to the site of tissue injury.\textsuperscript{24,29–32} This results in the formation of matrix bone formation and angiogenesis by stimulating increased levels of VEGF.\textsuperscript{32} This may lead to accelerated soft-tissue healing because of neovascularization.\textsuperscript{32}

In a study designed to evaluate the effectiveness of PRP when added to a xenograft, Aghaloo et al\textsuperscript{33} was able to demonstrate an increase in bone formation. Using immunohistochemical analysis, they found an increase in expression of PDGF, TGF-b and basic-fibroblast growth factor at 1, 2, and 4 months with the addition of PRP to the graft material.

Cytokines involved in connective tissue repair and bone regeneration are TGF-b1 and TGF-b2.\textsuperscript{24,26,34,35} Their most important role appears to be chemotaxis and mitogenesis of osteoblast precursors and stimulation of deposition of type I collagen matrix during wound healing.\textsuperscript{34,35} In vitro and in vivo studies have also shown that TGF increases cell proliferation of mesenchymal stem cells and osteoblasts leading to regeneration of bone. Specifically, TGF-b2 has been shown to increase osteoblast and osteoclast activity. An increase in TGF-b2 may accelerate bone regeneration by controlling the activity of osteoblasts and osteoclasts.\textsuperscript{36,37}

The main action of fibroblast growth factor (FGF) is to promote angiogenesis during vascular penetration
into bone; it is released by neutrophils, lymphocytes, monocytes and macrophages.26,38 Animal and human clinical studies have demonstrated that FGF stimulates osteoblastic proliferation and regulates extracellular matrix production. All of these processes are involved in bone formation and wound healing.39,40

Like FGF, VEGF is involved in angiogenesis.24,26,41 It stimulates the flow of nutrients, antibodies, and immune competent cells by chemotraction.24,26 VEGF has been identified as an endothelial cell-specific mitogen and chemotactic.42,43

**Rationale for HBO therapy**

The beneficial effect of oxygen in maintaining normal tissue homeostasis and promoting wound healing of both soft and hard tissues is critical. Bone formation, neoangiogenesis, fibroblast proliferation, and collagen synthesis are all influenced by specific cytokines (growth factors) and oxygen tensions in the tissues.44,45 Marx and Johnson46 demonstrated that HBO has proven to be effective in the management of osteoradionecrosis, which is an ischemic necrosis of bone caused by high-dose radiation treatment. The mechanism of ORN is a compromised vascularity and cellularity, as neocellularity and fibroplasia leading to synthesis of hard and soft tissues are impaired, especially bone and periosteum.46 The result is a wound that fails to heal, as the metabolic demands surpass current oxygen tension levels in the soft tissues. The diagnosis is based on the clinical signs and symptoms of pain, a nonhealing wound with soft-tissue breakdown (ulceration or necrosis of the mucous membranes), and exposure of necrotic bone persisting for greater than 12 weeks.47,48

HBO induces neovascularization and has been shown to revascularize the soft tissues of the maxillofacial region reversing the hypocellular, hypocellular, and hypoxic conditions due to radiation treatment by creating steep oxygen gradients between radiated and normal tissues. The steep oxygen gradient will stimulate macrophage-derived angiogenesis growth factor and macrophage-derived growth factor to the site of wound injury. Both of these growth factors will promote capillary budding and collagen synthesis during neoangiogenesis.44–46

It has also been shown that oxygen under hyperbaric conditions acts synergistically with VEGF to stimulate capillary budding and bone metabolism.49,50 The efficacy of HBO therapy in patients with ONJ has not been fully established, as clinical trials of the benefits of HBO treatment are still under investigation.8–10,13–15,17 During hypoxic conditions, oxygen deprivation occurs because of ischemia, which appears to signal release of VEGF. Downregulation of VEGF could be caused by hypoxic-inducing factor-1, which has been detected in bone cells.51,52 It is believed that HBO stimulates upregulation of VEGF by inducing synthesis of prostacyclin and nitric oxide.53

PDGF is a naturally occurring cytokine produced by macrophages, fibroblasts, and keratinocytes that stimulates fibroblasts to secrete extracellular matrix. It has been shown that PDGF-BB acts synergistically with HBO in managing nonhealing diabetic ulcers.54 In a rabbit study, Zhao et al55 showed that HBO combined with PDGF-BB or TGF-b1, was able to reverse the ischemic effects on soft tissues.

**PRP Preparation Technique**

Approximately 55 mL of whole blood from each patient was obtained from the antecubital fossa of the arm via venipuncture using a 23-gauge needle. The blood is collected and anticoagulated with an anticoagulant citrate dextrose-A solution. The venous blood is then injected into a disposable dual chamber, which is placed into a cell separator. Over a 13-minute period, the blood is processed using a microprocessor controlled, automated cell separator (Harvest Technologies, Plymouth, Mass). At a speed of 5600 rpm, the cell separator divides the venous blood into three components: PRP, platelet-poor plasma, and red blood cells.

Centrifugation of 55 mL of whole blood results in 10 mL of PRP. Next, 5000 units of bovine thrombin (King, St Louis, Mo) is mixed with 10% calcium chloride (American Regent, Shirley, NY). The entire 10 mL of PRP is topically sprayed onto the surgical site and activation of PRP results in degranulation of the platelets and immediate release of its growth factors.

**Case Reports**

**Case report No. 1**

The patient is an 84-year-old Asian woman who was referred to the oral and maxillofacial surgeon (CYSL) in January 2006 for evaluation of a nonhealing oroantral communication of the left maxillary sinus that was present for 14 months (Figure 1). Past dental history was significant for implant treatment in January 1999. The left maxillary sinus was grafted with allogeneic particulate bone followed by placement of 3 threaded dental implants without event. In August 2004, the patient began to develop chronic peri-implantitis around the implants. Attempts to control the condition failed and, because of progressive bone loss, all of
FIGURES 1–5. FIGURE 1. Large oroantral communication in left buccal vestibule of posterior maxilla in patient No. 1. Patient reported chronic retrograde flow of liquid and food from oral cavity out through the left nostril for past 14 months. FIGURE 2. Computerized tomography (CT) scan. Coronal view of patient No. 1 demonstrating near complete opacification of the left maxillary sinus. Destruction of the alveolar crest of the maxilla and inferior portion of the lateral wall of the maxillary sinus is observed. CT scan obtained with in-office cone beam iCAT (Imaging Sciences International, Hatfield, Pa). FIGURE 3. (a) Low-power view demonstrating nonvital, necrotic bone. Colonies of filamentous bacteria identified as *Actinomyces* (arrows). Hematoxylin-eosin stain. (original magnification ×40) (b) High-power magnification demonstrating filamentous bacteria identified as *Actinomyces*. *Actinomyces* means, "ray fungus," but the pathogen is not a fungus. It is a Gram-positive, non-spore-forming bacteria that can grow as filaments or rods. (original magnification ×400) FIGURE 4. Oroantral communication of the left posterior maxilla successfully closed by rotating a palatal-based pedicled flap harvested from the palatal shelf in patient No. 1. Adjunctive therapy consisting of platelet-rich plasma and hyperbaric oxygen were used to revascularize the soft tissues to stimulate wound healing and neoangiogenesis. FIGURE 5. Postsurgical iCAT cone beam CT scan (Imaging Sciences International) 9 months after surgery. Coronal view demonstrates resolution of sinus opacification of left maxillary sinus.
the implants were eventually lost. Necrotic bone was observed in the left posterior maxilla due to non-healing of the gingival tissues. Multiple attempts were made to debride the posterior maxilla of necrotic bone and to close the exposed alveolus, but this proved difficult. As the condition worsened, an oroantral communication developed. Further attempts were made to cover the exposed bone and close the oroantral communication, which failed.

Upon initial examination in January 2006, the past medical history was significant for osteoporosis. Current medications prescribed by the patient’s physician included Fosamax over a 9-year period. The patient reported no allergy to medications and denied using tobacco or alcohol. Oral examination of the left posterior maxilla demonstrated a large oroantral communication in the buccal vestibule. Exposed necrotic bone was observed along the alveolar crest of the lingual cortical plate of the maxilla. Because of chronic infection, it was observed that the buccal cortical plate was partially eroded. A CT scan was obtained, which was suggestive of chronic osteomyelitis of the maxilla (Figure 2). However, malignancy could not be ruled out.

After consulting with the patient’s physician, the Fosamax was discontinued in preparation for surgical treatment, and HBO treatment was initiated using the Marx protocol. The patient underwent 20 HBO dives at 2.4 atmospheres of absolute pressure for 90 minutes before any surgical procedure and 10 HBO dives at the conclusion of surgical treatment. Biopsy of the exposed alveolar crest of the palate of the maxilla was performed and submitted for histopathologic examination. The biopsy specimen demonstrated necrotic bone colonized by inflammatory cells and filamentous bacteria identified as Actinomyces (Figures 3A and 3B). The microscopic diagnosis was consistent for osteonecrosis of the left posterior maxilla and zygoma. Because of the Actinomyces bacteria, intravenous penicillin therapy was initiated for the next 3 months. The patient was informed of the clinical findings and the differential diagnosis included BP-associated ONJ, chronic nonsuppurative osteomyelitis, and actinomyces osteomyelitis.

Before closure of the oroantral communication, the sinus was debrided under direct vision through the oroantral communication. Two weeks later, the patient was taken back to the operating room, and the oroantral communication was successfully closed by rotating a large palatally based pedicle flap over the defect. Next, PRP was prepared according to the manufacturer instructions (Harvest Technology, Plymouth, Mass) and 10 mL of PRP was topically sprayed on to the surgical site, especially to the soft tissue flap to enhance wound healing and stimulate neovascularization. After 3 months of intravenous penicillin, oral penicillin V potassium (penicillin VK), was prescribed to the patient for another 3 months. The patient continued to return for follow-up observation, and at 9 months after surgery, necrosis of the palatal flap was not observed at any time (Figure 4). In addition, a CT scan was obtained, which shows complete resolution of sinus pathology (Figure 5). After successful treatment of the oroantral communication, the patient has elected not to resume BP use for her condition of osteoporosis.

Case report No. 2

The patient is a 76-year-old Caucasian man who was referred to the oral and maxillofacial surgeon (CYSL) in March 2006 for evaluation of a nonhealing surgical wound for the past 5 months. The patient’s dental history was significant for allogeneic block graft surgery in September 2005 in preparation for implant surgery. Two weeks after bone graft surgery, the bone graft site became infected with soft-tissue wound dehiscence. In an attempt to salvage the bone graft, the surgical site was debrided, and the patient placed on oral penicillin VK. However, because of necrosis of the graft and nonhealing of the gingival tissues, the necrotic bone graft was removed. For the next 5 months, it was observed that the soft tissues failed to heal by secondary intention with chronic exposure of bone and pain.

Upon initial examination in March 2006, the past medical history was significant for hypertension, chronic pulmonary obstructive disease (COPD), pneumonia, and osteoporosis. Current medications prescribed by the patient’s physician included Fosamax. The patient reported no drug-related allergies and no history of tobacco or alcohol use. Oral examination of the left posterior mandible revealed necrotic alveolar bone that was exposed and mildly painful to digital inspection. Plain radiography (Figure 6) and CT demonstrated an osteolytic pattern in the left posterior mandible that had extended near the inferior border of the mandible. The CT scan features were consistent with chronic osteomyelitis (Figure 7).

Under local anesthesia, biopsy of the exposed alveolar crest of the mandible was performed and submitted for histopathologic examination. Microscopic analysis revealed necrotic bone with empty lacunae (Figure 8). It was also observed that the necrotic bone was colonized by inflammatory cells and filamentous bacteria, which included Actinomyces...
spp. The microscopic diagnosis was consistent with osteonecrosis of the left posterior mandible.

The patient was informed of the clinical findings, and the differential diagnosis included BP-associated ONJ, chronic nonsuppurative osteomyelitis, and actinomyces osteomyelitis. After consulting with the patient's physician, the Fosamax was immediately discontinued in preparation for surgical treatment. In addition, an infectious disease specialist was consulted and intravenous penicillin therapy was initiated over a 3-month period.

Before initiating surgical treatment, the patient's physician was consulted to discontinue the BP therapy for a period of 3 months. Unlike patient No. 1, HBO therapy was contraindicated because of his condition of COPD. Therefore, only PRP therapy was included during the surgical debridement. After the mandible was debrided, 10 mL of PRP prepared according to the manufacturer instructions was sprayed onto the debrided mandible and soft tissues. Primary closure of the buccal and lingual gingiva and mucosa of the mandible was then successfully accomplished. The postoperative course was uneventful, and the patient remained on the intravenous penicillin for an additional 3 months. After the intravenous penicillin was discontinued, the patient was placed on oral penicillin VK for an additional 3 months. Although it was recommended to resume BP therapy, patient No. 2 had elected not to resume BP therapy for his osteoporosis. Follow-up observation continues and at 6 months after surgery, no evidence of dehiscence or wound breakdown was observed. A postoperative panoramic radiograph was obtained that demonstrates remodeling of bone (Figure 9).

**DISCUSSION**

Reports documenting difficulty in management of ONJ have been linked to the use of intravenous and, most recently, oral BP drugs. It is hypothesized that ONJ is caused by a disruption in the osteoblast-osteoclast homeostatic cycle, which leads to exposed bone in either the maxilla or mandible. However, we hypothesize that use of these therapeutic agents may also cause a hypovascular response of the jaws and surrounding soft tissues that leads to tissue hypoxia.
and ischemia. Although the pathogenesis is still uncertain, specific cytokines and cell populations, such as vascular endothelial cells, fibroblasts, and mesenchymal cells, are likely affected by BP drugs. The wounding of these specific target cells is a compromised vascularity. Therefore, the goal of treatment is to stimulate a new blood supply to the affected area.

We have described 2 patients with ONJ. Both patients were prescribed Fosamax for osteoporosis. The duration of oral BP therapy in these 2 patients was 5 to 9 years. Patient No. 2 did have a history of receiving recent (over a 12-month period) dental treatment. Patient No.1 did not have a history of recent dental surgery but suffered from peri-implantitis of the implants in the left posterior maxilla. The osteonecrosis that developed around the implants is an example of spontaneous ONJ.

Intraoperatively, it was difficult to identify definitive bleeding margins of bone during surgery. Therefore, the surgery in each patient was limited to removing nonvital, mobile bone sequestra. After removing as much nonvital bone as possible, the exposed jaw in each patient was successfully closed by primary closure or rotation of a pedicled flap without postoperative dehiscence or wound breakdown. In both patients, periosteal integrity was preserved while mobilizing the gingival and mucosal tissues.

In patient No. 1, PRP and HBO were used, and we theorize that the success of treatment is possibly attributable to the synergistic vascular effects of both treatments. In patient No. 2, only PRP was used, but we still managed to obtain a successful outcome of elimination of pain and primary wound closure. Based on our experience of bone graft reconstruction procedures, we believe the use of PRP may have a reparative effect at the site of tissue injury that could lead to elimination of pain and successful closure of exposed bone with or without the addition of HBO.

It is hypothesized that the altered cellularity, vascularity, and hypoxia observed in ONJ is due to underexpression of VEGF and endothelial cell apoptosis.24–26 Fournier et al demonstrated that BP agents inhibit vascular endothelial cell proliferation and function. The BPs have also been demonstrated to cause an increase in endothelial cell apoptosis and a reduction in revascularization.22

The VEGF is a potent cytokine that has been found in autogenous cancellous bone and a strong stimuli of VEGF secretion is hypoxia.22,60 In early wound healing, oxygen deprivation occurs and stimulates glycolysis to increase energy production. This cascade of events increases VEGF levels, which may in turn increase vascular permeability and stimulate the formation of new blood vessels.61

In several studies, PRP has been shown to increase VEGF levels, and upregulation of this specific growth factor may be responsible for revascularization of the bone matrix and soft tissues.24,25,41,61 This in turn, could be responsible for complete closure of the soft tissues over the exposed jaws without wound breakdown resulting in successful remission of ONJ.

In the podiatric medicine literature, Barrett demonstrated the successful use of PRP on chronic open wounds of the foot. Patients were included in the study if any foot ulcers or wound dehiscence failed to heal by other treatment method for 4 weeks or longer. By incorporating PRP in treating chronic open wounds of the foot, complete wound closure was obtained in 16 of 17 patients for a 94% remission. In another podiatric medicine study using PRP, Tsang demonstrated that EGF has a similar effect as VEGF on chronic foot ulcers.

Currently, HBO is not recommended in the management of ONJ, but this treatment is still under investigation.8,9,15,23,64 However, it has been shown that HBO can stimulate angiogenesis and fibroplasia, reversing the hypovascular and hypoxic conditions of ORN and actinomycosis.44–50 It increases both the oxygen tension and oxygen gradient in the soft tissues of the wound, which stimulates healing.

In a case series reported by the Duke Center for Hyperbaric Medicine and Environmental Physiology at Duke University Medical Center, they reported complete remission in 64% of intravenous-associated ONJ patients using HBO. In this study, the criteria of complete remission was defined as complete re-growth of the oral mucosa over the previously exposed bone, plus cessation of pain. In both of our patients, we were able to successfully obtain complete remission of ONJ. In patient No. 1, HBO proved clinically beneficial. Debridement and pedicled flap surgery was completed, with complete remission and without flap failure. We believe the physiologic effects of HBO (neovascularization and reperfusion) have the same effects in the ONJ patient as in the radiation-induced ORN patient, which can reverse the tissue hypoxia.

Currently, biopsy of the lesion is recommended only when attempting to rule out a metastatic lesion.23 However, we prefer to perform this procedure routinely, not only to rule out a metastatic lesion. Without identifying the pathologic condition and specific microorganisms, we cannot effectively manage the condition with confidence. In the Marx series of case reports, they demonstrated high numbers of...
Researchers have commonly reported the presence of Actinomyces spp, but the clinical significance of this observation has not been established.

Successful treatment of patients with ONJ is a definite challenge to the clinician. Both of the patients presented in this study were refractory to all treatment protocols such as surgical debridement and long-term antibiotic therapy before the use of HBO and PRP. We believe ONJ is not solely attributable to supression of osteoclast activity. Rather, osteonecrosis of the jaw not only involves alteration of the osteoblast-osteoclast homeostatic cycle, but is also multifactorial in origin, with a specific vascular component. Therefore, our rationale for implementing PRP and HBO was evidence-based scientific knowledge of these 2 therapeutic strategies in promoting angiogenesis and reducing the hypoxic effects of compromised hard and soft tissues from irradiated tissues, infection, and maxillofacial reconstruction.

We recommend the following adjunctive treatment protocol in an ONJ patient who is being treated for osteoporosis with oral BPs: If possible, cessation of BP therapy for 3 months or longer before surgical intervention; conservative surgical debridement of the jaws; intravenous and oral penicillin-type antibiotic therapy; 0.12% chlorhexidine oral rinses to control secondary infections of soft and hard tissues with good oral hygiene; and use of PRP and HBO. However, in cancer patients treated with BPs, hyperbaric oxygen treatment is contraindicated as HBO has a stimulatory affect.

Although our sample size was only 2 patients and long-term data are lacking, based on our results of successful remission (complete soft tissue coverage), we recommend that each patient discontinue oral BP therapy for at least 3 months or longer before surgical intervention. Before discontinuing BP therapy, consultation with the patient’s physician is mandatory. In cases of malignancy, discontinuing BP therapy may not be possible, and all comorbidity issues must be considered. Concurrently, we recommend that intravenous antibiotic therapy be implemented at least 3 months before surgical intervention and continued for another 3 months, especially if Actinomyces bacteria are one of the pathogens identified.

Numerous case reports of ONJ have identified the genus Actinomyces in their hard-tissue biopsy specimens. Researchers have commonly reported the presence of Actinomyces in ORN patients. They attributed the presence of these anaerobic, filamentous bacteria to a surface contaminant and not as the cause of ORN. In 2005, Store, Eribe, and Olsen and Store and Olsen using DNA-DNA hybridization technology observed several bacterial species in 9 of 14 resected radionecrotic mandibles, including Actinomyces. Scanning and transmission electron microscopy identified bacterial species, including rods, spirochetes, and cocci deep in the marrow spaces. One specimen also contained yeasts organisms.

Although Actinomyces is a normal inhabitant of the oral cavity, this significant finding deep in the marrow suggests that this bacteria could be of importance in the pathogenesis of ORN, rather than previously thought of as a surface contaminant.

The presence of microorganisms deep in the marrow strongly suggest an infectious etiology responsible for ORN. This new finding may require further research into the relationship of osteomyelitis and ORN.

Actinomycosis is not a fungal infection, but it is a slow-growing, non-spore-forming Gram-positive bacterial infection that demonstrates pleomorphism. The bacteria can grow as filaments, branching rods, and diphtheroidal rods. These pathogens are anaerobic or microaerophilic and are sensitive to antibacterial therapy and not antifungal medications. Studies have demonstrated that Actinomyces is chemotactic, activates lymphocytes, and stimulates the release of lysozomal enzymes from polymorphonuclear leukocytes and macrophages. For Actinomyces to become active, it requires the presence of other types of bacteria that can survive in an anaerobic environment. The specific role of these other types of pathogens remains unclear. Actinomycosis occurs when Actinomyces bacteria are introduced into tissues with a compromised vascular supply. The infection usually involves soft tissues, but it has been reported to spread to bone in 15% of cases. Diagnosis of actinomycosis is confirmed by isolation and identification of the pathogen. Actinomyces should be considered when a Gram stain of purulent discharge demonstrates Gram-positive filaments, rods, or diphtheroids with or without branching.

In both of our patients, Actinomyces bacteria were identified. Like ORN, it can be debated that this observation does not represent a surface contaminant but is part of another mechanism in the pathophysiology of ONJ. Therefore, treatment included a prolonged course of intravenous and oral antibiotics. Actinomycosis produces a necrotic infection that results in a hypovascular tissue, which prevents satisfactory penetration of antibiotics into the infected wound. In studies by Curi et al and Hansen et al, the presence of Actinomyces-positive tissue proved difficult to cure, as these bacteria promote a chronic and inflammatory environment that can lead to
development of orocutaneous fistulas and refractory osteomyelitis.

Management of this difficult infection requires conservative debridement of the necrotic bone, usually by curettage and meticulous soft tissue closure. High-dose antibiotic therapy for several months is indicated. Curi et al reported that treatment time of ORN was significantly prolonged in patients with Actinomyces bacteria compared with those who had Actinomyces-negative tissues (29.7 months versus 13.4 months). Intravenous penicillin G, 3 to 12 million units daily is recommended, followed by an oral antibiotic regimen of Penicillin VK, 500 mg 4 times per day for an additional period of 3 months or longer.

**CONCLUSION**

Currently, there is no consensus and success in the management of patients with ONJ, including patients taking the oral form of BP drugs. In a typical dental practice, we believe clinicians will encounter far more patients prescribed the oral form of BP medications from their physician to manage the effects of osteoporosis.

An adjunctive treatment protocol with vascular considerations that use PRP and HBO has been presented in 2 patients receiving long-term oral BP therapy for osteoporosis. Local delivery and upregulation of specific growth factors to the site of a nonhealing wound caused by oral BP therapy using PRP, with and without HBO, may stimulate angiogenesis and reperfusion leading to complete remission of the nonhealing osteonecrotic wound. Because of the small sample size of only 2 patients in our study, PRP and HBO will remain a questionable adjunctive treatment strategy in ONJ patients being treated for osteoporosis. Further studies with a larger patient database are needed to fully evaluate the potential positive effects of this adjunctive treatment in ONJ patients taking the oral form of BPs.

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**REFERENCES**


