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Regeneration of Mandibular Osteoradionecrosis Defect with Platelet Rich Plasma Gel

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Abstract. *Osteoradionecrosis (ORN) of the mandible is a major complication of radiation therapy of head and neck cancer with a potential of occurrence ranging from 5 to 15% of the irradiated patients. Due to the gradual necrotic process, the mandibular bone becomes necrotic and looses its spontaneous regeneration ability. Containing an elevated content of mitogenic and osteogenic growth factors, the use of platelet rich plasma (PRP) from autologous source has been suggested to re-activate the healing process of osteogenesis. Autologous PRP gel was introduced into the ORN necrotic defect of a 44-year old patient previously treated for squamous cell carcinoma of the tongue, subsequent to proper surgical debridement. We report post-operative two-year follow-up demonstrated by panoramic X-ray which showed regain of the mandibular bone continuity with a complete repair of the necrotic defects. We conclude that this case illustrates an incident of successful regeneration of ORN critical-sized defect of the mandible by autologous PRP gel.*

Radiation therapy is a treatment modality used in a wide variety of maxillofacial cancer, either as a primary approach or in conjunction with chemotherapy and surgery. Despite this modality of cancer treatment, it causes changes in the skeletal system by decreased bone vascularity and repair capacity, nonetheless it is still preferred as the treatment of choice for the sake of eliminating the malignant neoplasm.

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Mandibular osteoradionecrosis (ORN) is a potentially devastating complication of radiation therapy of head and neck cancer. Radiation has been proven to impair the function of osteoblasts, thus decreasing bone matrix production. Due to its superficial location and poor vascularization the mandible is particularly prone to this undue complication. Osteopenia is the peculiar x-ray finding of ORN and is typically seen one year after irradiation (1). ORN has been reported in many studies to occur with an incidence following radiation therapy ranging from 5 to 15% (2). Patients suffering from poor dental health show a higher risk for this side effect. Systemic promoting factors may also include tobacco or alcohol consumption, malnutrition and a poor general condition. Recently, treatment schedules with bisphosphonates therapy have been included among risk factors as they may inhibit angiogenesis and induce apoptosis apart from their virtuous role in inhibiting of osteoclasts and treating bone resorptive disease (3, 4). Being affected by the necrotic progressive process, the mandibular bone undergoes degradation of the roots leading to teeth exfoliation, rendering a suitable environment for possible infections leading to a source of severe pain.

Proper vascularisation is well known to be a primary requirement for bone regeneration and repair (5-8). In 1983, Marx proposed a protocol for management of ORN of the mandible based on a vascularization improvement strategy (9). This protocol consisted of a treatment modality starting from a conservative approach with the aim to eventually reduce the need of radical resection and reconstruction of the mandible. In fact the non conservative treatment has an elevated potential for adverse side effects and functional sequalea. The suggested conservative approach was based on the use of selective antibiotic therapy associated with hyperbaric oxygen (HBO) therapy enhancing wound healing through an increase in tissue

oxygen tension, resulting in vascular proliferation and healthy bone regeneration. Patients with extensive ORN or in whom non-invasive therapy was ineffective often require aggressive resection and reconstruction associated with a microvascular composite flap. The procedure was based on the introduction a non-irradiated blood supply into the region, thus improving the chance for wound healing and bone viability (10-13). Both conservative approaches include the regime of a long term antiseptic/antibiotic treatment scheduled for at least 10 weeks after ORN resolution. Considerable time and resources are required afterward to manage ORN by these approaches, especially in patients who had been free of cancer for many years (3, 14).

Recent studies, report the possibility of bone regeneration by using platelets rich plasma (PRP) with promising results (15-20). PRP is the portion of blood containing the concentrate of platelets which are rich in mitogenic growth factors (GF) such as platelet derived growth factors (PDGFs), transforming growth factor beta TGF-β, epidermal growth factor EGF, insulin like growth factor IGF and vascular endothelial growth factor VEGF. Growth factors entrapped within the alpha granules in the platelets corpuscles are released upon a process called 'platelets activation' that consists of bursting the granules to release their GF content. This occurs primarily by thrombin. Within their environment of release, such GF play a crucial role in orchestrating the molecular cascade of healing. The benefit of the introduction of such GF into a healing lesion might be emphasized especially in critical-size defects. These defects, by definition, bypassed a certain size beyond which the natural healing process becomes compromised where the tissue destruction cannot be reversed into regeneration. The therapeutic advantage of PRP in this type of defect is the introduction of the 'right' concentrated GF that are missing for the healing process and their introduction in natural proportions necessary for a proper interaction to stimulate the different pathways that ultimately lead to the activation of gene expression and production of the necessary proteins for healing (21, 22).

In this case report, we investigated the effect of autologous PRP for regeneration of the mandibular necrotic defects in a cancer patients who previously underwent radiation therapy. We speculated that the concentrated mitogenic and vascular enhancing GF within the PRP would improve bone regeneration in the necrotic defects by recruiting vessels and enhancing cell migration from the healthy surrounding bone tissue.

Case Report

A white Caucasian male age of 44 was diagnosed for oral squamous cell carcinoma (SCC) of the left half of the tongue. The procedures of diagnosis, surgical intervention and follow up were carried out in the Institute of Cancer Research IST- Genova and were approved by the Institutional Ethics Committee.

A partial left glossectomy was performed with conservative neck dissection (CND) and bilateral suprahyoid lymph node dissection. Pathological staging of the tumor was PT1pN0. Following surgery, the patient received adjuvant radiotherapy (RT). The schedule consisted of 33 visits, 5 visits per week; each consisted of a dose of 200 cGy with a total dose of 6600 cGy. Lonidamine - an indazole carboxylic acid that has been shown to be synergistic with radiotherapy- was added to the radiotherapeutic protocol (23). After four years, the patient showed up edentulous, with stomatitis, purulent abscesses and exposed alveolar processes of the right mandible. He reported xerostomia and teeth exfoliation during the previous years. The panoramic X-ray showed diffuse alveolar resorption together with mandibular radiolusencies thus a diagnosis for ORN was subsequently made (Figure 1A). Pus bacterial culture evidenced the presence of *Prevotella melaninogenica* and *Prevotella buccae* (isolated from anaerobic culture) and *Streptococcus anginosus*. The patient was treated with Moxifloxacin Hydrochloride 600 mg daily dose for 20 days, subjected to regular oral antisepsis and eventually scheduled for regenerative surgery with autologous PRP. During surgery, the necrotic bone was removed and the PRP gel was introduced into the defect area. A panoramic X-ray was performed two years later (Figure 1B).

Platelets rich plasma gel preparation. Patients were hosted at the Immunohematology Centre – St. Martino Hospital, Genoa, Italy two days before surgery. A quantity of 450 ml of whole blood was collected and immediately centrifuged at 200g for 30 minutes to separate packed red blood cells and PRP. The red blood cells were reinfused to the patient while the PRP was re-centrifuged at 2000g for 5 minutes to precipitate the platelets and separate the platelets poor plasma (PPP) from the platelets concentrate (PC). PPP was immediately frozen at –80˚C in a mechanical refrigerator then at 4˚C for 18 hours for spontaneous thawing while the PC was preserved at 22˚C in continuous agitation.

With regards to the quality control of the product, PC had a platelet count equal to 60×10^9, residual leucocytes equal to 0.2×10^9, and maximum volume of 30 ml. The cryoprecipitate had factor VIII equal to 70 μl/100 ml, fibrinogen equal to 140 mg/unit and maximum volume of 30 ml. Autologous thrombin was prepared from 27 ml of blood collected into three sterile tubes containing 1ml of acid-citrate-dextrose (ACD) anticoagulant solution. Tubes were centrifuged at 900g for 10 minutes and then plasma was transferred into a second sterile tube. One ml of sterile calcium chloride was added to each 5ml of plasma. The tubes were incubated for 30 minutes at 37˚C. The plasma clot was then re-centrifuged for 10 minutes at 900g. The supernatant thrombin was stored at –30˚C until the time of use.

At the time of surgery, equal volumes of PC and cryoprecipitate were mixed in a sterile Petri's dish and 1ml of thrombin and 1 ml of calcium gluconate were added to each

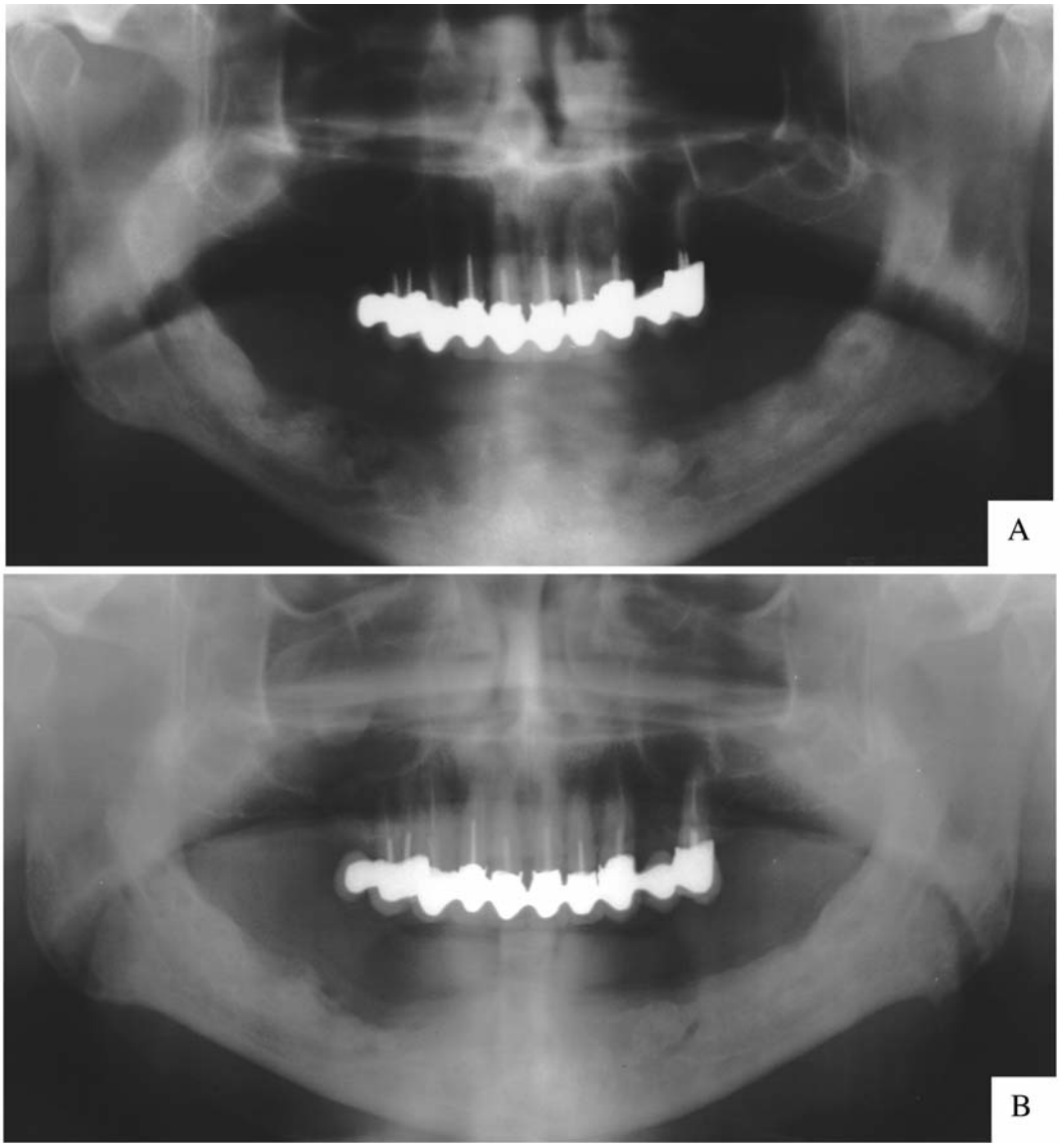

Figure 1. *Panoramic X-ray films. A. Pre-operative panoramic radiograph shows multiple radiolucent and sclerotic areas with poorly defined borders in the right mandible extended to the left mandible as well as recent extraction sockets. B. Post-operative panoramic radiograph shows regeneration of the bone and regain of the alveolar bone mass after reconstructive surgery.*

10 ml of the PC- cryoprecipitate mixture. The gel resulted ready for application after 10-15 minutes of adding thrombin and calcium ions.

Results

The regenerative treatment with PRP did not show any complications; the patient was discharged the day after the surgical procedure. There was no evidence of postoperative infection or any oro-mandibular fistulae. The mandibular bone repaired completely after being covered by mucosa with evidence of surgical wound healing within 8 postoperative days.

After the reconstructive surgery, the 2-year follow up panoramic radiograph showed complete regain of the alveolar bone mass and mandibular bone regeneration in the place of the large irregular radiolucency (Figure 1 A and B).

Discussion

Mandibular osteoradionecrosis is an aggressive complication of head and neck radiotherapy. Its management requires thorough surgical debridement together with HBO application, microvascular reconstruction or a combination of both (13). These integrated treatment modalities require considerable

time and expenses. Some studies reported HBO to be of doubtful benefit while a second reconstructive procedure could be needed as a result of flap failure (13, 24-26).

Recently, relevance has been given to the use of platelet-rich plasma (PRP) alone or in combination with bone graft materials by obtaining bone regeneration through bioengineering (22, 27-31). In this study, we report successful restoration of mandibular integrity and continuity of an advanced ORN defect based solely on the debridement of the lesion and the regenerative potential of the PRP without concomitant HBO therapy. The reported case involved an advanced deterioration of the necrotic process with bone sequestration which then lead to the exposure of the alveolar process with associated superimposed infection (32).

Based on the poor vascularisation of the mandibular bone, the destructive effect of the radiation on the bone forming cells cannot be simply faced by bone deposition through new osteogenic cells recruitment that would otherwise occur in highly vascularized tissues. Progressively, the necrotic process compromises the integrity of the mandibular trabecular structure leading to bone sequestration and teeth exfoliation thus favouring an environment for infection. Introducing the PRP rich in mitogenic and chemotactic factors into such a necrotic defect was expected to be the best simulation to a natural healing process that might occur on the basis of an adequate vascularization. During tissue healing process, blood platelets are the main source to release necessary GFs for this process. Upon vessel injury, the platelets stick to the subendothelial tissue collagen proteins and form the platelets plug that stabilizes the clot within the fibrin meshwork. Platelets are activated by the thrombin involved in the cascade of healing by releasing their polypeptides content crucial for signalling the local mesenchymal and epidermal cells migration, division and consequently collagen and glycosaminoglycans synthesis. PDGF is a potent mitogen and chemotactic factor for osteogenic cells. It stimulates bone collagen synthesis in addition to its angiogenic properties. It is chemotactic to polymorphonucleocytes, macrophages, fibroblasts, stimulates the production of fibronectin molecules necessary for cellular adhesion, migration and hyaluronic acid that helps wound contraction with remodeling. TGF-beta stimulates the proliferation of osteoblasts precursors together with stimulating bone collagen synthesis and it has a direct action on the differentiation function of the osteoblasts. It was found to favor bone formation by inhibiting the formation of osteoclasts in addition to its angiogenic, chemotactic and anabolic functions. Insulin-like GF enhances new bone formation by increasing the expression of type I collagen and increasing the rate of matrix deposition. In combination with PDGF it enhances the rate and quality of wound healing. VEGF is involved in the process in which new blood vessels invade devascularized tissue and is mitogenic to endothelial cells (33-38). Understanding the interaction between such factors and their environment allows the opportunity to understand the molecular cascade of events that would help us to recapitulate the natural healing process if it had been compromised. Furthermore, it should be emphasised the role of adequate debridement to eliminate necrotic bone and to allow the introduced GF to recruit osteogenic cells and vessels from the healthy exposed bone. The complex issue of the role of such factors in orchestrating the healing process is encountered as each GF may be involved in different regeneration pathways and a single pathway may involve several GF (22, 39).

In this case report, a successful regeneration of the mandibular integrity was obtained by introducing the platelets GF into the necrotic defect mimicking the natural healing process otherwise compromised by inadequate vascularization. To our knowledge, this is the first study that reports beneficial effects of PRP on ORN defect healing with a two-year follow up period. In this case, we did not test if the ORN was resistant to HBO (Marx stage III disease) thus we cannot assume that PRP is effective in such case. We should eventually remark that the patient had edentulous mandible after the regenerative procedure, which might have also reduced complications that usually occur in the presence of periodontal pathologies or septic foci.

In conclusion, we believe that the use of PRP associated to conservative strategies for ORN treatment may reduce or eliminate the need for invasive procedures such as the resection and reconstruction of the mandible with vascular flaps.

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