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Efficacy and safety of medical ozone (O₃) delivered in oil suspension applications for the treatment of osteonecrosis of the jaw in patients with bone metastases treated with bisphosphonates: Preliminary results of a phase I–II study [☆]

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SUMMARY

Osteonecrosis of the jaw (ONJ) is an adverse event that has been reported in patients receiving cancer treatment regimens, including bevacizumab, bisphosphonates, and denosumab. We performed a preliminary open label, prospective phase I–II study in patients treated with bisphosphonate to evaluate the treatment effect and tolerability of medical ozone (O₃) delivered in an oil suspension on BONJ lesions ≤ 2.5 cm. Ten consecutive patients with BONJ lesions not responsive to conservative treatment were pre-treated with 10 days of antibiotics to reduce purulent secretions on the gum. The exposed bone lesion and osteomucosal edge was cleaned with an ultrasonic scaler. The BONJ lesion was treated with 10 local applications of medical O₃ delivered in an oil suspension for 10 min. In all patients, mucosal lesions resolved with complete reconstitution of oral and jaw tissue, with 3–10 applications. No toxicity was reported. Unexpectedly, total sequestration of the necrotic bone, with spontaneous expulsion in eight patients and new bone formation around the necrotic area in two patients was observed. No patient required surgical intervention. In two patients with pre- and post-treatment X-rays, no residual bone lesions were observed after treatment. These preliminary results show the efficacy and tolerability of O₃ delivered in an oil suspension applied directly to BONJ lesions ≤ 2.5 cm, thus indicating that BONJ can be a manageable and potentially curable condition.

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Introduction

The occurrence of bone metastases is a common event in cancer patients, with increased risk of skeletal-related events (SREs) such as pathological fractures, spinal cord compression, the need for orthopaedic surgery and/or radiotherapy to the bone, pain and hypercalcaemia.^{1–3} The role of bisphosphonates (BPs) in the prevention of SREs, that significantly impact on the quality of life of patients with either solid tumours or multiple myeloma, has been well established.^{1–12} Consequently, BP therapy has been incorporated into clinical practice recommendations for these patient populations.^{13–19}

Osteonecrosis of the jaw (ONJ) is an uncommon but sometimes devastating adverse event, which has been reported in patients receiving complex cancer treatment regimens including chemotherapies, BPs and RANKL inhibitors and anti-angiogenic agents.^{20–26} The reported incidence of ONJ ranges from 1.2% to 9.9%, mostly derived from retrospective analyses.²⁷ The weight prevalence was 13.3% for studies which documented follow-up.²⁸ The exact aetiology of ONJ is still unclear.²⁷ Concomitant risk factors include tooth extractions (in about 60% of cases), invasive dental surgery during the course of anti-resorptive therapy and duration of the therapy. Poor oral hygiene, concurrent disease (e.g. diabetes, peripheral vasculopathy) and use of concomitant cancer drugs such as chemotherapy, thalidomide, and corticosteroids may be additional risk factors.^{20–24,27}

However, as animal studies showed ONJ development in dogs exposed to BPs in the absence of dental surgery/infections, more research has to be performed on potential ONJ causes²⁹ and clinical diagnosis.

Several medical and dental associations and expert panels have published clinical practice recommendations for dental preventative measures.^{20,30–33} Recent studies carried out in patients with

[☆] The procedures followed in this study were reviewed and approved by the Ethics Committee of National Cancer Institute of Milan and are in accordance with the ethical standards of the Helsinki Declaration (1964, amended in 1975, 1983, 1989, 1996 and 2000) of the World Medical Association.

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solid tumours³⁴ and multiple myeloma³⁵ support these published clinical recommendations, thereby highlighting the importance of a close collaboration between oncologists and dentists for the early identification of patients at risk to develop ONJ, and the importance of an experienced dental staff to assess the patient. Appropriate preventive dental measures may lead to a reduction of ONJ incidence in up to 70% of solid tumours³⁴ and an almost three-fold decrease in patients with multiple myeloma.

However, a careful evaluation is mandatory because at least 30% of patients treated with BPs and developing ONJ (BONJ) may present with the non-exposed variant of BONJ and these cases would be missed in studies using a 6-week exposed bone criterion.

Unfortunately, clinical recommendations for ONJ treatment are lacking. It has also been demonstrated that aggressive dental surgery often worsens bone exposure in patients with ONJ. Therefore, a conservative approach consisting of intermittent prophylactic antibiotic therapy, rinses with oral chlorhexidine and careful sequestrum removal is recommended.^{20,27,28,30–33,36} The efficacy of hyperbaric oxygen therapy in the treatment of ONJ is controversial.³⁴

Ozone is a gas naturally produced by atmospheric air; medical ozone is produced from oxygen. Ozone has antimicrobial and wound-healing properties. The role of O₃ produced by air to treat ONJ has been evaluated in some pre-clinical and clinical studies because it was thought that O₃ could induce the repair of tissue by cleansing the osteonecrotic lesions, which leads to mucosal healing.³⁷

In animal studies of rats and dogs with infected apical periodontum, positive results were obtained with the use of local O₃-perfluorane complex. This treatment resulted not only in reduced inflammatory infiltration, but also in the complete resolution of destructive manifestations to the bone.³⁸ Ozone has been shown to enhance the benefits of surgical and pharmacological treatments of ONJ when administered as a gas before and after dental treatment procedures in patients with multiple myeloma who developed ONJ during treatment with BPs.^{39–42} O₃ can exert positive effects on the bone lesion by oxidative preconditioning, stimulating and/or preserving the endogenous antioxidant systems and by blocking the xanthine/xanthine oxidase pathway for reactive oxygen species generation.

Some authors have used O₃ as a gas medication in the treatment of patients with avascular necrosis of the jaw because of its biological reaction stimulating effects, its influence on oxygen metabolism, its antibacterial properties and, finally, because it does not damage the tissues, but helps to restore normal bone physiology.^{39,41} The efficacy of O₃ gas has been investigated in conjunction with antibiotics and surgery^{39–42} and showed only limited efficacy.

In this uncontrolled open-label study, we investigated the effect of ozone in the treatment of BONJ that failed to respond to antibiotics through the assessment of partial or complete curative response and tolerability of localised topical application of an oil

suspension enriched with medical O₃ gas, produced from pure oxygen, as the treatment for ONJ lesions ≤ 2.5 cm. The use of this oil, which was not applied on healthy tissue, was approved by Italian Ministry of Health. For the treatment, we used a prototype of a medical device whose cost was about 100 Euro for 100 g of gel, sufficient for 20 applications. We investigated the outcome in ten consecutive patients who developed ONJ following BP therapy and whose ONJ lesion did not heal with prior conservative therapy with various cycles of antibiotics.

Patients and methods

Patient characteristics

Table 1 shows the patient demographics and baseline disease characteristics. Ten patients (2M/8F, mean age 65, range 53–77) with malignancies involving bone metastases who developed ONJ lesions were included in the study. Nine patients were previously treated with zoledronic acid, and one with pamidronate; none of them had received recommended preventive dental measures or was previously treated with radiotherapy to the head-and-neck nor to the jaws. In eight patients, the ONJ lesions appeared in the area of a previous dental extraction and in the remaining two patients in the dentures plate area. The minimum number of BP infusions before the diagnosis of ONJ was two in one patient undergoing zoledronic acid infusion and five in one patient after pamidronate infusion. The ONJ lesions were unsuccessfully treated with antibiotic therapy in all patients and two patients also received hyperbaric oxygen therapy. At the time of this investigation, no patient reported spontaneous ONJ healing.

ONJ was diagnosed by an experienced maxillofacial dentist on the basis of the following criteria: presence of exposed bone in the maxillofacial region with no evidence of healing after 6 week of appropriate dental care.⁴³ No patients had bone metastasis at the jaw or osteoradionecrosis after OPT and CT studies.³³

All patients included in this study presented with a single ONJ lesion of relatively small size (width ≤ 2.5 cm, depth ≤ 0.5 cm), nine in the mandible and one in the maxilla. The lesions were classified according to Weitzman³³ as follows: 60% were 3A, 20% were 2A and 10% each were 4A and 4B (Table 1). Median time between the ONJ diagnosis by the Dental Team and the start of the investigational therapy with O₃ oil was 360 days (range: 0–790). The study was reviewed and approved by the Institutional Ethical Committee, the Declaration of Helsinki was adhered to and all patients provided written informed consent before entering the study.

Procedure

Although antibiotic therapy had been received frequently by the patients prior to study entry, all the patients were pre-treated

Table 1
Patient demographics and baseline disease characteristics.

Pt.	Age	Gender	Primary cancer	ONJ classification**	Time (days) from ONJ diagnosis to first O ₃ oil application
1	74	F	NHL	3A	728
2	54	F	Breast	4B	175
3	77	F	Breast	3A	199
4	73	M	Prostate	4A	0
5	70	F	Breast	3a	14
6	55	M	Multiple Myeloma	2A	108
7	53	F	Breast	2A	61
8	71	F	Multiple Myeloma	3A	20
9	59	F	Breast	3A	790
10	62	F	Breast	3A	0

F, female; M, male; NHL, Non-Hodgkin Lymphoma.

** ONJ classification according to Weitzman et al.³³

with antibiotic therapy (azithromycin 500 mg/day) for 10 days prior to the initiation of the medical O₃ oil treatment. In addition to antibiotic treatment, the exposed bone and osteomucosal edge were cleaned with a tartar supersonic scaler in order to reduce the infective component at gum level and favour the penetration of O₃ oil through the mucosa around the ONJ lesion. An experienced dentist applied the O₃ oil suspension in situ, directly on the ONJ lesion area using a patient customised silicone device.

The O₃ oil suspension was maintained at a temperature of at least 4 °C (status of gel). Contact of the O₃ gel with the infected oral mucosa at body temperature led to a change of state from gel to oil, and a release of ozone gas, which interacts with the necrotic tissue. Surgical dentistry suction was performed both during and after each application to avoid ingestion of the oil suspension, to prevent the inhalation of O₃ particles produced during the exothermic reaction, and to avoid potential local and systemic toxic effects.

According to the study protocol:

- Each patient was treated with a maximum of 10 applications of O₃ oil once every 3 days,
- Each O₃ oil application lasted 10 min,
- The treatment was stopped when patients showed intolerable adverse effects,
- Patients who did not show a clinical response and whose wound showed exposed bone but clean edges were eligible for surgical resection of the necrotic bone, rotation of the mucosa lap, and surgical joining of the two edges.

Assessments

Efficacy and safety evaluations were performed after each O₃ oil application and during the follow up period scheduled at 1, 2, 3, 4, 6 and 8 months after the completion of the treatment.

Partial curative effects were considered: marked reduction of the infective status and mucosal edge of the lesion progressively healed. The infective status with purulent secretions is not evaluated as an integral part of the ONJ lesion, but rather as a superinfection complication due to the accumulation of the alimentary plaque and increase of microbial biofilm.

In the partial curative response, an improvement of the mucous tissue trophism is achieved but the necrotic bone is not sequestered and surgical dissection of the skeletal fragments is still needed.

Complete curative effects were: (1) total or partial sequestrum of necrotic bone to be surgically removed, (2) healed and reepithelialised mucosa with regenerated epithelial tissue.

Safety criteria were: odour intolerance, burning mucosa, coughing due to local irritation of respiratory tract, dysphagia post treatment, symptoms related to skin sore or mucosal lesion.

The treatment area was assessed for the presence or absence of the following possible side effects of therapy: mucosa redness oral around the lesion area, pain, progressive increasing of lesion, appearance of necrotic area, petechiae and/or bleeding.

The treatment was stopped when patients showed clinical response or undesirable adverse effects. Moreover, pain intensity was assessed at each visit by means of a self-reported numerical rating scale (NRS).

Study design and statistical considerations

This was a single-centre, open label, Simon two-stage optimal design study.⁴⁴ The primary efficacy end point of the study was the complete clinical response rate, calculated as the proportion of patients showing a complete response – defined as complete healing of the mucosal lesion – over the total number of patients accrued. During stage 1, an enrolment of nine patients was re-

quired. If no response was observed, then the study was terminated. If at least one response was observed, the trial could continue to stage 2 and an additional 15 patients enrolled. After completion of the second stage of the study, the treatment would be considered worthy of further investigation if at least three responses were observed. The study design yielded a >90% probability of a positive result if the true response rate was >25%, and a >90% probability of a negative result if the true response rate was <5%. The study design incorporated monitoring of treatment associated toxicity, with a Bayesian stopping rule in case of a 90% posterior probability of a toxicity rate greater than 10%.⁴⁵

Results

Efficacy

Complete clinical response with resolution of all the damage was achieved in all patients. Complete response was achieved after three applications of O₃ oil in three patients, four applications in four patients and 10 applications in three patients, with a mean recovery time of 27 days (range: 7–78) (Table 2).

Figure 1 shows the pictures of a lesion at the different time points from T0 (diagnosis of ONJ, before start of therapy, 1A) to the time of complete wound healing with re-epithelialisation of oral mucosa (1F). Unexpectedly, 80% of patients developed bone sequestrum with spontaneous expulsion of the necrotic bone, which was removed by the dentist with an anatomical pincher (Fig. 1D). This procedure was performed without pain or bleeding. The mucosal and gum tissue underlying the area of the bone sequestrum was completely healed and re-epithelialised with regenerated epithelial tissue. It is of interest that the surgical intervention planned in the protocol to dislodge the necrotic bone was

Table 2
Patients' outcomes with medical O₃ in oil suspension therapy.

Pt.	Tn (number of medical O ₃ applications and Time (days from 1st application) to healing)	Clinical outcome
1	T4 (10)	Bone sequestrum Spontaneous bone expulsion* re-epithelialisation**
2	T10 (78)	Bone sequestrum Spontaneous bone expulsion* re-epithelialisation**
3	T4 (10)	Bone sequestrum Spontaneous bone expulsion* re-epithelialisation**
4	T4 (10)	Bone sequestrum Spontaneous bone expulsion* re-epithelialisation**
5	T10 (78)	Bone sequestrum Spontaneous bone expulsion* re-epithelialisation**
6	T3 (11)	Bone sequestrum Spontaneous bone expulsion* re-epithelialisation**
7	T3 (7)	Bone sequestrum ossification*** re-epithelialisation**
8	T10 (30)	Bone sequestrum ossification*** re-epithelialisation**
9	T3 (11)	Bone sequestrum Spontaneous bone expulsion* re-epithelialisation**
10	T4 (20)	Bone sequestrum Spontaneous bone expulsion* re-epithelialisation**

* Expulsion, of the necrotic bone.

** Re-epithelialisation, of gum mucosa.

*** Ossification: new bone formation around the necrotic area.

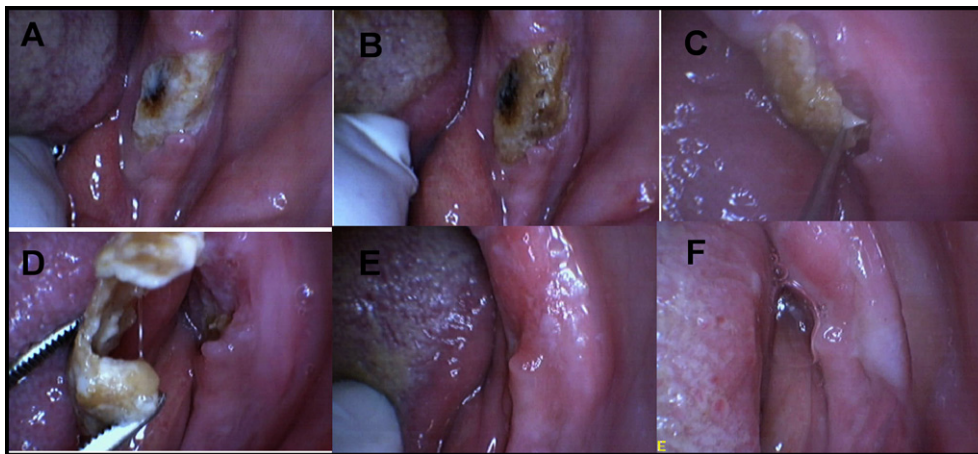


Figure 1 From beginning of therapy with medical O₃ oil to complete removal of bone sequestrum and reepithelisation. Reports a clinical case at different time points from T0 at diagnosis of ONJ, before start of therapy (A) to the time of complete wound healing with reepithelialisation of oral mucosa (F). This patient presented at T0 with an osteonecrotic lesion of 11.09 × 8.5 mm (A). As per protocol, the patient was pre-treated for 10 days with antibiotics (Azitromicine, 500 mg/day) and then with four applications of medical O₃ oil. The osteonecrotic bone sequestrum was easily removed by the dentist using an anatomical pincer, without pain or bleeding (D). The mucosal tissue underneath the bone sequestrum was completely healed and reepithelialised with regenerated epithelial tissue (E-F). Pathological examination of the necrotic bone sample confirmed the clinical evidence of necrosis.

not necessary in any of the patients, because this necrotic bone was spontaneously expelled.

In two patients (20%), the formation of new bone (ossification) around the necrotic area followed by mucosal re-epithelialisation was observed. In addition, in two patients with a radiological examination pre- and post-treatment with O₃ oil, we observed the disappearance of the bone lesion with complete reconstitution of oral and gum tissue within 8 months of follow-up. In all patients, the following biological changes of the gum's mucosa were observed during the healing process: (1) a marked reduction of the infective status of the patient with healing after 10 days of antibiotic therapy and a complete regression of purulent secretion, also due to the cleaning action by the tartar supersonic scaler; (2) progressive improvement of tissue trophism and increased regeneration of the tissue integrity, with spontaneous expulsion of the necrotic tissue fragment in 80% of the patients; (3) the healing of the exposed area and the re-epithelialisation of oral mucosa, without the spontaneous expulsion of necrotic tissue, was progressively attained in two patients. The applications were stopped when the mucosa of the lesions were completely healed.

No recurrences of ONJ were observed in any of the ten patients treated during 8 months of follow-up from the start of O₃ oil therapy. After completion of the treatment, all patients were able to eat normally and prosthetic dentures were adapted and re-positioned for those patients who had dentures.

Safety

No patients reported adverse events and no objective or subjective symptoms of intolerance to the O₃ oil applications were observed. All patients indicated that the smell and taste of the O₃ oil preparation was distasteful.

Discussion

Our study is the first to demonstrate healing of ONJ lesions by means of a therapy based on medical O₃ gas delivered in an oil solution directly to ONJ lesion <2.5 cm. This is an easy-to-use and original method, in the absence of observable toxicity and without the need for surgical bone removal. The main differences

between our study and the previous experiences reported in the literature are the following:

- We used medical O₃ produced from medical rather than atmospheric oxygen. This made it possible both to preserve the antimicrobial and wound-healing property of O₃ and to avoid the presence of toxic molecules such as oxide, hydrocarbon contamination and others.
- The medical O₃ was applied directly to the lesion, as an oil suspension enriched with the medical O₃ gas.
- The O₃ oil suspension was applied in situ, placed in direct contact with the ONJ lesion using a silicone device, customised for each patient.
- Antibiotic therapy was administered before starting the O₃ oil applications with the aim to clean the secretions on the gum. In published studies, administration of O₃ and antibiotics was concomitant, and its role was mainly to enhance the antibiotic therapy.
- 70% of the patients treated needed less than ten applications of O₃ delivered in an oil suspension.
- Finally, and most importantly, the patients enrolled in our study did not undergo any invasive dental procedure or surgery.

Our new and original therapeutic approach for the clinical management of small necrotic maxillary lesions in ONJ was based on a non-surgical intervention for the application of O₃. This approach consisted of in situ positioned O₃ oil suspension to increase the therapeutic effect of O₃ and to reduce the risk of infection or inspiration in the oral cavity. Consequently, this approach showed a good safety profile. Antimicrobial treatment before starting the O₃ oil applications plays a critical role in reducing the severity of inflammation and infection. Moreover, it is important to cleanse the exposed wound and osteomucosa edge with a tartar supersonic scaler, to support the penetration of the O₃ through the tissue.

In conclusion, the preliminary results of this study demonstrate that medical O₃ delivered in an oil suspension should be considered a promising, effective, safe and simple therapeutic option for the treatment of small ONJ lesions.

However, the most important limitation of our study is the uncontrolled nature. It is important to stress that the major

aim in presenting the data of this pilot uncontrolled study is to inform researchers and stimulate them to perform further higher quality (RCTs) multicentre trials to improve the quality of the results.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.oraloncology.2011.01.002.

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