Geranylgeraniol (GGOH), incorporated into a bone cement pellet promotes osteoclast function and healing in a model of medication-related osteonecrosis of the jaw[☆]

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ABSTRACT

Introduction: There is no cause -based treatment for Medication-Related Osteonecrosis of the Jaw (MRONJ). MRONJ is a morbid condition including exposed, infected bone and mandibular fractures in osteoporotic individuals and metastatic cancers patients treated with nitrogen containing bisphosphonates (NBP). NBPs inhibit farnesyl diphosphate synthase (FDPS) in the mevalonate pathway, depriving osteoclasts and other bone cells of small GTPases necessary for their function and survival. We test the hypothesis that geranylgeraniol (GGOH),a metabolite downstream of FDPS, when incorporated into a bone cement pellet, enhances osteoclast function and promotes local bone healing in *in vitro* and in a proven animal model of MRONJ.
Methods: ^{3}H labelled GGOH (2 mM) was incorporated into a Hydroset bone cement pellet and release from the cement was assessed over time. To assess the effect on bone cell function, the GGOH-loaded cement was placed in a porous filter above cultured osteoclasts treated with bisphosphonate and the effect on osteoclast survival and function were measured. In a pilot study the effect of GGOH on osteotomy microstructure was measured in a rat model of MRONJ using a split mouth design.
Results: The release of GGOH from bone cement increased osteoclast survival/metabolic activity, and promoted resorption of the calcified substrate. In vivo released GGOH limited the effects of the bisphosphonate and promoted healing. In an animal pilot study, GGOH from the infused cement carrier stabilizes bone structure and restores the ability of osteoclasts to remodel.
Conclusion: These initial findings point to GGOH in a bone cement carrier as a useful therapeutic approach to prevent or mitigate the pathogenesis of MRONJ.

1. Introduction

Nitrogen containing bisphosphonates (NBPs) are currently prescribed for osteoporosis, osteogenesis imperfecta, Paget's disease, multiple myeloma and patients affected by metastases from breast, prostate and other cancers.[1] One side effect of these drugs is the development of medication-related osteonecrosis of the jaw. (MRONJ). The American Association of Oral and Maxillofacial Surgeons defines MRONJ as a pathologic condition in which exposed bone or bone that can be probed through an intraoral or extra-oral fistula(e) in the maxillofacial region

that has persisted for 8 weeks in patients who have been treated with anti-resorptive or anti-angiogenic agents who have had no history of radiation therapy.[1] No treatment for this debilitating disorder currently exists based on its etiology. Current theories suggest that inhibition of angiogenesis, infection, local trauma and oral mucosal toxicity might be causal.[2,3] The most accepted hypothesis concerning MRONJ etiology focuses on NBPs suppression of osteoclast function and bone turnover.[3,4] By inhibiting farnesyl diphosphate synthase (FDPS), an enzyme in the mevalonate pathway that generates cholesterol and isoprenoids, NBPs suppress the prenylation of small GTPases vital for osteoclast maturation

Fig. 1a. Kinetics of release of GGOH from Hydroset pellets. A combination of ^{3}H labelled GGOH and unlabeled GGOH was added to the bone cement carrier to achieve a total concentrations 2 mM. The carrier was incubated with 0.5–1.0 ml PBS at 37C on a rocking platform. At 0.5–96 h, media was removed, counted. With a powder to liquid ratio of 2.5:1.0, after 96 h, 92 % of the loaded GGOH was released into medium. At each time point, 3 medium aliquots were counted and averaged.

and function.[5–7] Geranylgeraniol (GGOH) is a metabolite downstream of FDPS that rescues epithelial cells, fibroblasts and osteoclasts from NBP toxicity in vitro.[8–12] It has also been shown to reverse the effect of MRONJ when injected systemically.[8] Approaches to administer the drug include the daily local administration of high concentrations of GGOH as a spray.[13] It may be argued that this type of application would be unsuitable for the patient, and an inefficient way of delivering this drug. Likewise, systemic administration of GGOH would negate the therapeutic effect of NBPs in non-oral tissues. Currently, there is no method to deliver clinically effective doses of GGOH in a controllable manner to tissues of the oral cavity. The purpose of this study is to test the hypothesis that GGOH when incorporated into a calcium phosphate cement pellet can reverse the toxic effect of NBPs in osteoclasts and maintain bone cell function in vitro. Additionally, using a proven animal model of BRONJ, we report, in a pilot study, that a GGOH infused bone cement pellet promotes the initial phase of local healing within mandibular bone.

2. Materials and methods

Cells and materials: Murine transformed macrophages (ATCC 264.7-TIB-71) were obtained from ATCC (TIB71) and cultured in DMEM with 10 % FBS. Initial stocks were grown for 4 cell doublings and frozen. Treated with recombinant mouse RANKL (R&D systems; cat #462-TR-010), these cells differentiated into osteoclasts over a 4–6 day treatment period. The NBP used for the study included Pamidronate (Sigma; cat #p2371-10mg) and Zoledronate. (cat #SML0223); GGOH was purchased from Sigma (cat #G3278-100 mg). Studies of the release kinetics of GGOH from the bone cement utilized, ^{3}H GGOH (all trans) Specific Activity: 50–60 Ci/mmol 1.85–2.22 TBq/mmol (American Radiolabel Chemicals).

Bone Cement Carrier: Calcium phosphate bone cement (Stryker Hydroset) was evaluated as a carrier for GGOH. For Hydroset, 5μl of 100 mM GGOH was added to a mix of 0.625 g Hydroset powder (8 μl GGOH/g powder) + 250μl liquid. This mix was sufficient to create 5 bone cement pellets and produced a concentration of 2 mM within each pellet and a liquid to powder ratio of 2.5:1. The pellet was allowed to harden after placing the mix in 4.7 mm diameter x 2 0.0 mm high sterile plastic tubing (Marina Air Line) for 30 min at 37C in a humidified chamber after which plastic tubing was removed. *In vitro* experiments were performed with the pellet suspended in 8 μM filter over cells attached to 24 well plates.

NBP toxicity experiments: $1.5–2.0 \times 10^4$ Raw 264.7 osteoclasts were grown on 24 well plates for 24 h, media was changed with fresh media
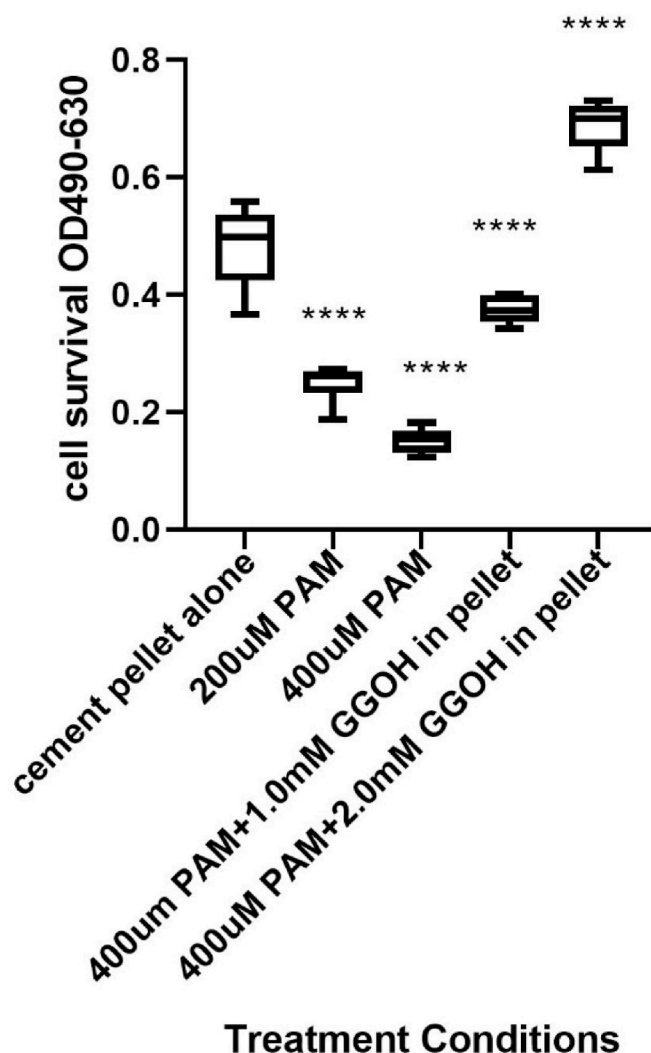
Fig. 1b. Geranylgeraniol (GGOH) maintains the viability of RAW osteoclasts treated with NBP. Murine transformed macrophages were grown on 24 well plates for 24 h, and then treated with 20 ng/ml of RANKL, in the presence of bone cement pellets containing 1.0 mM or 2.0 mM GGOH and in the presence of pamidronate (Pam, 400 mM). After 72 h, cell viability was quantitated, by measuring the change in optical density (OD) (****$p < 0.0001$).

containing 20 ng/ml of RANKL. Cells were treated with GGOH released from the bone cement pellets in the presence or absence of pamidronate for 72 h. Controls for the study included cells treated with the bone cement (no GGOH) only. The MTT assay was used to measure cell viability (Cell Titer 96 kit, Promega) and quantitated by measuring the optical density at 490 nm after subtracting background absorption at 650 nm. At each time point, 4–6 replicates were averaged.

Measurement of Osteoclast Function: Corning Osteo Assay 24 well plate (3987) was used to measure osteoclast function along with reagents from the Cosmo Bio Bone Resorption Assay kit (CSR-BRA-24). The assay is based on the osteoclast-dependent release into the conditioned medium of fluoresceinamine-labelled chondroitin sulfate which has been adsorbed onto a coated calcium phosphate surface. Resorption activity was evaluated by measuring the fluorescence intensity of the conditioned medium. Briefly, RAW cells were incubated for 96 h with or without GGOH and pamidronate in the presence of 20 ng/ml RANKL and fluorescence intensity of the conditioned medium was measured with an excitation at 485 nm and emission at 535 nm. At each time point 4 replicates were averaged. No-treatment controls were included in the experiment.
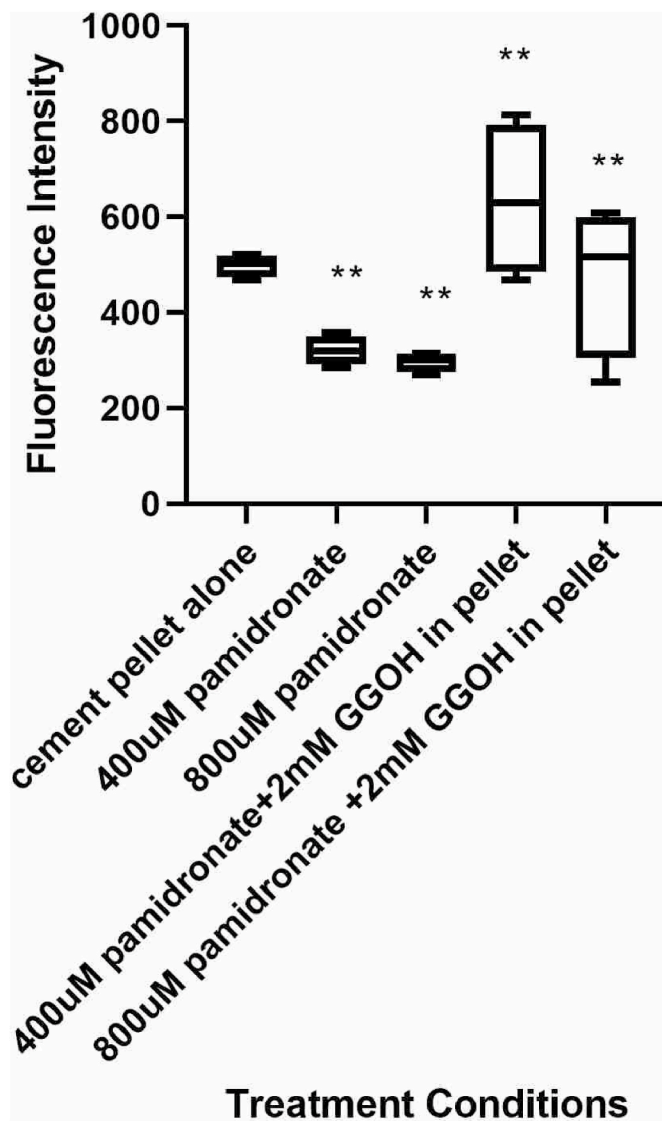
Fig. 1c. Geranylgeraniol (GGOH) supports the resorptive activity of RAW osteoclasts in the presence of pamidronate. Bone resorption activity of RAW osteoclasts was evaluated by measuring the fluorescence intensity of the dye released from fluoresceinamine-labelled chondroitin sulfate coated mineral into the conditioned medium. RAW cells were incubated with or without GGOH contained in a cement pellet and pamidronate in the presence of 20 ng/ml RANKL and fluorescence intensity was measured with an excitation at 485 nm and emission at 535 nm. At each time point, between 4 and 6 replicates were averaged. ($**p < 0.01$).

Kinetics of release of GGOH: A combination of ^{3}H labelled GGOH and unlabeled GGOH was added to the bone cement carrier to achieve a final concentration of 2 mM. The carrier was then placed in a scintillation vial filled with 0.5–1.0 ml PBS at 37C on a rocking platform. At selected time periods, media was removed, counted, and replaced with fresh media. The release of radioactivity from the 2.5:1 powder to liquid CaPO4 pellet (used in cell experiments) was measured by scintillation counting. Background counts were subtracted, and 3 replicates at each time period were averaged.

Animal studies: The study was approved by and performed in accordance with the guidelines of the institutional animal research ethics committee at Thomas Jefferson University (IACUC approval #02093-2018-08-28). Female 99 days old ovariectomized Sprague Dawley rats (n = 12) were purchased from Charles River Labs. Cages with filters were placed in a temperature-controlled room at 22C and 50

% humidity, with a 12 h light/dark cycle. The animals were fed a normal diet and water and acclimated for 1 week before beginning the investigation. After acclimatization, the animals were injected weekly with zoledronic acid (100 μg/kg, Sigma) for 10 weeks. Animals were anesthetized with ketamine (50–60 mg/kg), xylazine (5–7.5 mg/kg) and buprenorphine (0.05–0.2 mg/kg). The gingiva surrounding the surgical site was infiltrated with 0.1 ml 2 % lidocaine with 1:100000 epinephrine. After anesthesia was achieved, a full thickness gingival flap was prepared and osteotomies 1 mm in diameter x 1 mm deep were created using a #1 surgical round bur (S.S. White) at the mid-point of the mandibular diastema between molars and incisors on both the left and right sides using a battery driven hand piece. Saline irrigation was administered during this process. A Hydroset pellet containing 4.8 mM GGOH was inserted into the left side osteotomy. This pellet was created by mixing 12 μl of 100 mM GGOH with 0.625 g Hydroset powder (19.2 μl GGOH/g powder) + 250 μl liquid; a similar pellet, without GGOH was inserted into the right side osteotomy. A fresh batch of cement with or without GGOH was used for each animal. Both surgical sites were closed with resorbable sutures.

Microcomputed tomography (MicroCT) analysis: After sacrifice, the mandible was dissected from the rat skull. Connective tissue and epithelium were removed, and mandibles were fixed with 4 % paraformaldehyde (PFA) overnight in at 4C and stored in 70 % ethanol. The mandible was then bisected in between the mandibular incisors. Each half of the mandible (left that had received GGOH and right control) was used for MicroCT analysis (Skyscan 1275, Billerica, MA). The acquisition settings were voxel size 10 μm^3, with a tube voltage of 70 kVp and current 140 μA, and rotation step of 0.30. NRecon was set to a range standard of 0.066. Scans were performed along the long axis of the incisor, and transaxial and coronal images were taken. Morphometric measurements included tissue volume, bone volume, bone mineral density and closed porosity of the area 1 mm along the perimeter of the osteotomy defect.

Histomorphometric analysis: Samples were fixed in 4 % paraformaldehyde for 1 week and decalcified in 12 % EDTA at 4C for 4 weeks before embedding in paraffin. To facilitate histologic examination, each mandibular half was then further reduced by making bucco-lingual incisions 2 mm mesial and distal to the defect within the diastema isolating the osteotomies from the rest of the mandible. Each block containing the osteotomy was sectioned and alternate sections were H&E and TRAP-stained respectively. TRAP-stained sections were imaged and the number and localization of osteoclasts were determined by an automated macro (ImagePro Plus software) screening for the purple coloring using a size threshold. Two specific regions of interest were defined in each section and analyzed separately: 1) vascular bed below the defect, and 2) the area encompassing the defect. The osteoclast number was normalized by area to allow direct comparison.

Statistical Analysis: One way ANOVA analysis was performed using Prism software version 8.

3. Results

The goal of both the in vitro and in vivo studies was to utilize a bone cement that would release a quantity of GGOH that facilitates osteoclast function and stabilizes bone tissue structure. Fig. 1a shows that at a powder-liquid ratio of 2.5:1, after an initial rapid release phase lasting 1 h, GGOH release followed first order kinetics. By 96 h, 92 % of the GGOH was released from the cement pellet. This formulation was used for both the in vitro and in vivo studies.

For studies of osteoclast viability function, transformed macrophages (ATCC 264.7-TIB-71) were differentiated into functional osteoclasts when treated with RANKL. Exposure to 200 μM and 400 μM pamidronate for 72 h resulted in a 49 % and 69 % loss of viability (p < 0.0001) at each concentration, respectively. When 1.0 mM GGOH was included in the in the bone cement pellet, in cells exposed to 400 μM pamidronate, viability increased by 247 % (p = 0.0001). At this bisphosphonate
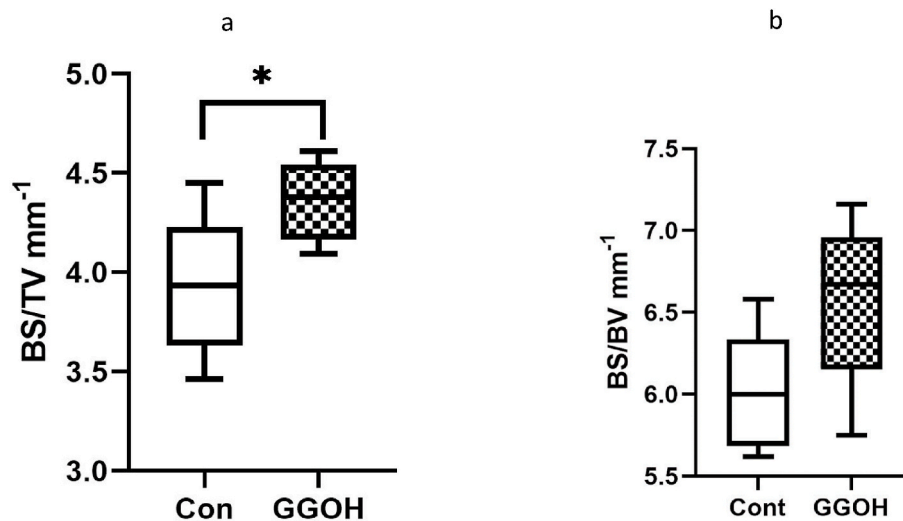
Fig. 2. MicroCT analysis of bisphosphonate treated mandibular osteotomies of rats treated with GGOH. Bone cement pellets were inserted into the right (control) and left (GGOH containing) osteotomies and evaluated by MicroCT analysis after 9 days. **(a)** Measurement of bone surface/trabecular volume (BS/TV). Note, the GGOH-treated side (left) was 10.94 % higher on the left side ($p = 0.0471$) than the control side. **(b)** Measurement of bone surface/bone volume (BS/TV) was 9.5 % higher on the treated side compared to the control ($p = 0.0798$).

concentration, an increase the GGOH concentration to 2 mM increased viability by 450 % ($p < 0.0001$). Bone cement alone had no significant effect on survival (Fig. 1b).

Since GGOH rescued osteoclasts from the toxic effects of pamidronate, we determined if this agent salvaged osteoclast function (Fig. 1c). Osteoclasts were exposed to varying concentrations of pamidronate and GGOH over a period of 4 days. To quantitate osteoclast function, fluorescent dye release from plates coated with mineral bound to fluoresceinamine-labelled chondroitin sulfate was measured. Concentrations of 400 μM and 800 μM reduced osteoclast function by 36 % ($p = 0.0128$) and 41 % ($p = 0.0037$) respectively. When treated with GGOH (2 mM) loaded-into Hydroset pellets, fluorescent dye release increased by 215 % for cells exposed to 400 μM pamidronate. Osteoclasts exposed to 800 μM pamidronate showed a 184 % increase in resorptive function. ($p < 0.01$) (Fig. 1c). Pamidronate had no effect on osteoclast function for doses delivered by bone cement that were 200 μM or less (results not shown).

MicroCT analysis was utilized to assess the effect of GGOH on healing bone in osteotomies performed on the rat mandible. Fig. 2a shows that nine days after bone cement pellets were inserted into the right (control) and left (GGOH containing) mandibular diastemas, the bone surface density/trabecular volume (BS/TV) on the left side was 10.94 % higher side ($p = 0.0471$). Likewise, Fig. 2b indicates that the bone surface density/bone volume (BS/BV) was 9.5 % higher on the treated side compared to the control ($p = 0.0798$). The closed porosity, a measure of bone healing, was 88.7 % higher on the left side than the right ($p = 0.097$) (data not shown). After 9 days the treated side of the rat mandible showed signs of healing compared to the control with smoother osteotomy walls and vascularity. Control side lack of healing is evidenced by jagged walls of the osteotomy, empty lacunae and few blood vessels (Fig. 3a).

Trap-stained cells in the vasculature surrounding the defect were seen. Below the defect the GGOH treated side shows more TRAP-stained cells than control. Histomorphometric quantitation of cell size and percent of field occupied by cells in vasculature under the defect suggests larger cells occupying a greater area (Fig. 3b and c) This trend of greater healing continued in the animals sacrificed at 19 days with GGOH treated side showing smoother osteotomy walls more blood vessels and fewer empty lacunae. (Fig. 4a). After 19 days, below the osteotomy site, there were 280 % more TRAP-stained cells on the GGOH treated side than on control side. ($p = 0.0794$) (Fig. 4b). Moreover, close

to the osteotomy, the diameter of TRAP-stained cells, on the treated side were on average 158 % larger than controls ($p = 0.0120$). (Fig. 4c).

4. Discussion

It is reported here that a commercially available bone cement can serve as a vehicle to supply a metabolite that restores viability and function to osteoclasts exposed to physiologic levels of nitrogen-containing bisphosphonates. This metabolite, geranylgeraniol (GGOH), located downstream of farnesyl diphosphate synthase (FDPS) in the mevalonate pathway has previously been shown to rescue osteoclasts, oral fibroblasts and keratinocytes from cell death when freely available in cell culture medium.[7] Results herein show that this metabolite incorporated into a bone cement carrier rescues RAW cell derived osteoclasts from the toxic effects of NBPs and restores their function. Confirming the in vitro study, we show that in vivo, using NBP and ovarectomized animals to simulate post-menopausal osteoporosis, GGOH infused bone cement maintained bone structure in an osteotomy defect in the mandible and activated osteoclasts.

It is acknowledged that GGOH delivered topically to a tooth socket "locally" and daily for 35 days results in improved alveolar bone healing in a rat model of MRONJ.[13] However, this delivery method is impractical for human patients. Moreover, while GGOH delivered systemically in the rat model can reverse the effects of MRONJ this form of administration would likely negate the anti-metastatic effect of NBPs in patients receiving chemotherapy for breast, lung and other cancers. In view of these drawbacks, there is a need to develop an osteoconductive, resorbable and tunable carrier that could be used as a vehicle to deliver GGOH locally to avoid these systemic effects.

Notably, calcium phosphate resorbable cements have been used to deliver antibiotics, analgesics, anticancer, anti-inflammatory drugs and growth factors.[14,15] Results presented here show that GGOH can be released from a bone cement over a periods of 72–96 h in sufficient quantity and activity to rescue osteoclasts from the toxic effects of pamidronate and to restore the ability of osteoclasts to resorb a calcium phosphate substrate (Fig. 1). The kinetics of release of GGOH is affected by the ratio of powder to liquid (p/l) incorporated into the cement pellet. While a number of formulations were considered, our release study indicated that a ratio of 2.5:1 (p/l) caused a slow release of the drug over as 96 h time period. During this time period, there is rapid bone destruction in the mandibular osteotomies (Fig. 1).

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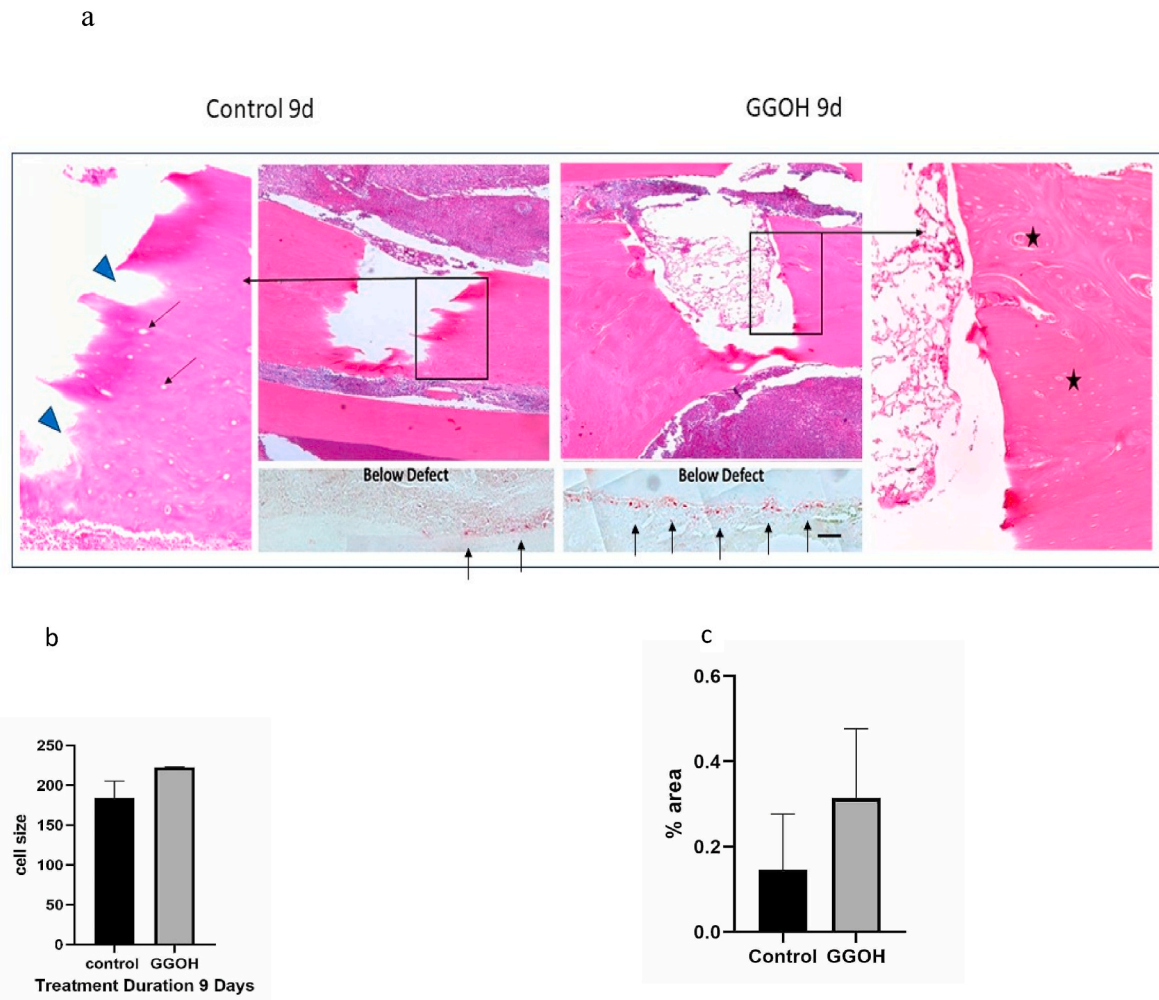
Fig. 3. Histologic and TRAP analysis of bisphosphonate-treated rat mandibular osteotomies following filling with the GGOH bone cement at 9 and 19 days and stained with H & E. **(a)** Left two panels shows a longitudinal section through the control (no GGOH) mandibular bone after 9 days. Note, the bone that lines the control osteotomy site exhibits a jagged morphology. Mag x 40. A higher power view of the boxed area (far left panel) shows that the jagged bone surface at the periphery of the osteotomy (arrowheads) contains many empty large cell lacunae (arrows) Mag x 100. Right two panels indicate the histology of the osteotomy site filled with bone cement containing GGOH. In contrast to the control, the bone walls of the osteotomy site are smooth suggesting that some healing is taking place. Mag x 40. The higher power view of the mandibular bone shows fewer empty lacunae and blood vessels (stars). Mag x 100. Bottom panel: TRAP staining of bone cells below the osteotomy defect. TRAP-stained cells appear more numerous on the GGOH treated side (right) than control. (left). (arrows) Mag 100x (magnification bar is 500um for 40x and 250um for 100x for all micrographs **(b)** and **(c)** show histomorphometric measurement of TRAP stained cell size and area occupied respectively. Both parameters suggest larger and more diffuse TRAP stained cells in the GGOH treated side of the rat mandible.

The release of 2 mM GGOH from the chosen bone cement formulation fully restored both viability and function to osteoclasts in the face of a 400 µM pamidronate challenge. Further, this concentration of GGOH was able to rescue osteoclasts treated with a toxic concentration of pamidronate (800 µM) (Fig. 1). This finding confirms earlier studies that show that GGOH restores TRAP (tartrate resistant acid phosphatase) staining, and other markers of osteoclastic differentiation.[8] Additionally, GGOH promotes protein prenylation function in osteoclasts exposed to zoledronate.[8,12] Based on the studies reported herein and results of investigations published elsewhere it is plausible to consider that the GGOH contained in bone cement would enhance the activity of osteoclasts, a critical component of the bone basic multicellular unit.

To determine if GGOH contained in bone cement would stabilize bone challenged with NBP, we used the technique introduced by Kim and Tadad, namely zoledronate-treated ovarectomized rats.[16,17] Ovarectomy simulates menopause in humans and is an inducing factor for MRONJ. Rather than extracting teeth from the animals which induces extreme pain and an inability to eat, an osteotomy was performed in the mandibular diastema. This method allowed the animals to retain their full dentition resulting in better post-operative survival, nutrition and health. Relevant to this model, tooth extraction is not a necessary MRONJ prerequisite as it is also generated by other forms of trauma.[18–20] Additionally, we chose to use a split mouth osteotomy design for the in vivo rat studies. This design reduced inter-animal genetic variability adding statistical power to the analysis of this small pilot study. Because it is widely used by other investigators in their in vivo modeling of BRONJ, zoledronate was used instead of pamidronate in our animal studies. Dunford and Thompson in 2001 showed that both drugs inhibit FDPS with zoledronate being 10x more potent than pamidronate.[6]

Histological analysis revealed there was stabilization of the mandibular alveolar bone at 9 days continuing through 19 days. (Figs. 3 and 4). This effect was most apparent at walls of the osteotomy in the areas of close contact with the cement pellet. In contrast, in the absence of GGOH, despite the presence of bone cement, the bone edges around the osteotomy are jagged with some empty osteocytic lacunae and relatively few blood vessels. The stabilizing effect of GGOH on osteoclasts was also evident: after 9 days, TRAP stained cells were larger and

Fig. 4. a) The bone that lines the osteotomy site at 19 days. The control bone (left panels) exhibits less of a jagged morphology than at 9 days. Mag x 40. A higher power view of the boxed area indicates that an incomplete surface lining is now visible (arrowheads) at the periphery of the osteotomy with empty cell lacunae. (asterix) Mag x 100. The osteotomy site filled with bone cement containing GGOH (right panel) is smooth suggesting that healing is taking place. Mag x 40. The higher power view of the mandibular bone shows fewer empty lacunae and more blood vessels (stars) Mag x 100. Bottom panel: TRAP staining of bone cells below the osteotomy defect at 19 days shows that GGOH treated bone exhibits a greater number of TRAP-stained cells compared to the control side. (arrows) **b**) and **c**) Histomorphometric analysis of TRAP-stained cells in osteotomy sites treated with and without GGOH at 9 days. Panel b shows that there were that there were 280 % more TRAP stained cells on the GGOH treated bone near the osteotomy site than the control side ($p = 0.0794$). Panel c shows that in terms of size, cells on GGOH treated side were on average 158 % larger than controls ($p = 0.0120$).

occupied a greater area on the GGOH treated side compared to the control side (Fig. 3); after 19 days, TRAP stained cells were significantly larger ($p = 0.0120$) and more numerous than on the control side. (Fig. 4). The presence of the larger TRAP stained cells suggests that GGOH's released from the bone cement is restoring bone homeostasis.

The MicroCT analyses confirm the histomorphometric studies. Experiments presented here show that GGOH when delivered from an osteotomy socket in the rat mandible can accelerate the process of bone formation in an NBP rat model of MRONJ. Nine days after GGOH infused cement implantation, the bone surface density within 1 mm of the osteotomy defect was 10.94 % ($p = 0.047$) greater than on the control side. The bone surface/bone volume measure and closed porosity measures were also supportive of increased bone healing (Fig. 2) These observations confirm earlier findings by Nagaoka and Kajiya that the effects of zoledronic acid on bone mineral density can be offset by intraperitoneal injections of geranylgeraniol and related metabolites of the mevalonate pathway.[8]

As was indicated earlier, the major goal of the study was to explore the notion that a commonly used bone cement can serve as a useful reservoir of GGOH for the treatment of osseous sites that are vulnerable to development of MRONJ in the post-menopausal state. While the findings from the pilot study indicate that the bone cement enhances bone stabilization and provides this carrier and release function, a number of experimental limitations need to be addressed. First, the study is of very limited duration. Clearly, it is desirable to extend the study beyond the first 19 days of treatment. In addition to longer term studies, further optimization of the GGOH-bone cement content needs to be established and, in many cases, related to the supra-pharmacological levels of NBPs used to treat cancer. Another limitation involves the MRONJ rat model itself. To minimize pain and suffering, which accompanies tooth removal and commonly associated with root apex retention, we opted for the split mouth osteotomy model. A better animal model would be minipigs which exhibit estrogen deficiency-related bone loss, oral microflora, extensive periodontitis and bone turnover values that are similar to those of humans.[21] Noteworthy, Tsutsumi and co-workers reported that when challenged by tooth extraction, these animals developed clinical and histopathological features of MRONJ.[22] Finally, a singular component of human MRONJ is bone infection and necrosis, presumably associated with an attenuated immune response. From this perspective the robust immunoreactivity of rodents limits the value of this animal species. Whether the bone cement could also be formulated to contain adjuvant and antibiotic agents is currently being

explored.

In conclusion, we have shown that bone cement can deliver a downstream metabolite in the mevalonate pathway that can counteract the inhibitory effect of NBPs on osteoclasts in vitro and restore function. In so doing it may be able to rescue osteoclasts from the toxic effects of NBPs and restore the ability of osteoclasts to resorb a mineralized substrate. In vivo this vehicle delivers GGOH to an animal model of NBP induced MRONJ in a manner that appears to accelerate the first signs of a healing response.

Declarations of interest

None.

Funding

Abbreviations

BS/TV	ratio of bone surface area divided by bone total volume
BS/BV	bone surface density/bone volume
GGOH	geranyl-geraniol
GGPP	geranyl-geraniol pyrophosphate
uCT	micro-computerized tomography
NBP	Nitrogen containing bisphosphonate
RANK-L	receptor activator of nuclear factor kappa beta ligand
TRAP	Tartrate resistant acid phosphatase

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