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Direct Pulp Capping in Osteoporotic Rats Treated with Zoledronic Acid

Osvaldo Zmener DDS[1], Cornelis H. Pameijer DMD, DSc, PhD[2*] and Roberto Della Porta DDS[3]

[1]Department of Specialized Endodontics, Faculty of Medical Sciences, University of El Salvador/Argentine Dental Association, Buenos Aires, Argentina.

[2]Department of Reconstructive Sciences, University of Connecticut, School of Dental Medicine, Farmington CT, USA.

[3]Department of Specialized Endodontics Faculty of Medical Sciences, University of El Salvador/Argentine Dental Association, Buenos Aires, Argentina.

***Correspondence:**

Cornelis H. Pameijer, Department of Reconstructive Sciences, University of Connecticut, School of Dental Medicine, Farmington CT, 2002 SW Laredo St, Palm City, FL 34990, USA.

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ABSTRACT

Background: *Osteoporosis is a metabolic bone disease, which impairs and/or delays the regenerative response of calcified tissues.*

Aim: *The aim of this study was to assess the histopathologic response to direct pulp capping in osteoporotic rats that received a preoperative treatment with Zoledronic acid, a bisphosphonate which is currently prescribed for treating osteoporosis.*

Methodology: *Two groups, each with 7 osteoporotic female Wistar rats (n=7) received either an intravenous dose of 100 mg/kg of Zoledronic Acid (Group 1) or the same volume of sterile saline (Group 2). One week later, the pulps of the right and left mandibular first molars of each animal were exposed and capped with Biodentine. After 20 days the animals in Group 1 received a second similar dose of Zoledronic acid and Group 2 a similar second dose of sterile saline. After 38 days the animals were euthanized and a total of 28 teeth (14 per group) were prepared for histologic analysis evaluating the following parameters: inflammation, reparative hard tissue formation, odontoblast cell layer organization, fibrous tissue, and necrosis.*

Results: *In Group 1 the reparative hard tissue formation was scarce with preservation of the pulp tissue structure and the odontoblast cell layer. In Group 2 the pulp was preserved as a fibrous reticular-like structure. Reparative hard tissue formation was totally absent and the odontoblast cell layer was atrophic or absent. In both groups, no inflammation or necrosis was observed.*

Conclusions: *The intravenous administration of Bisphosphonate (Zoledronic Acid) did not promote pulp healing in osteoporotic rats.*

Keywords

Biodentine, Direct pulp capping, Osteoporosis, Bisphosphonate, Zoledronic Acid.

Significance

Osteoporosis delays and/or impairs the regenerative response of calcified tissues. The administration of Bisphosphonates (BPN) is currently the therapy of choice for osteoporotic patients. This study shows that the administration of BPN such as Zoledronic acid did not contribute to the normal healing response after pulp capping in osteoporotic rats. Although this is a clinical test in rats, the results should be taken into consideration when osteoporotic patients are being treated for pulp capping procedures.

Introduction

Direct pulp capping (DPC) is a therapeutic treatment in which a protective agent is applied to a dental pulp exposed by traumatic/mechanical injuries or dental caries excavation. The objective of this procedure is to allow pulp healing to maintain pulp vitality and function, which is especially important in cases of immature young teeth. Experimental studies have shown that DPC has successfully been used in laboratory animals [1-3] while clinical studies in humans revealed similar results [4-6]. However, it is believed that pulp healing may be altered or impaired in patients with metabolic diseases. Osteoporosis (OPR) is a prevalent metabolic bone disease with increased susceptibility to bone fractures and a decreased expression of morphogenetic proteins. It is also thought to delay or impair the regenerative response of calcified tissues [7]. Studies in laboratory animals revealed that osteoporosis produced periodontal tissue atrophy and a significant reduction of the activity of osseous progenitor cells [8]. OPR is a common finding in adult patients, however, with some frequency it is also detected in young individuals [9,10]. In general, OPR occurs because of other systemic anomalies (secondary osteoporosis), which frequently are genetic in origin. OPR has also been reported in long-term corticosteroid therapies [11]. Various drugs such as Bisphosphonates (BPN) are used to treat osteoporosis. BPN increase hard tissue remodeling due to their regulating effect of hard tissue reabsorbing cell activity. Furthermore, BPN induce osteoblast differentiation and increase hard tissue formation [12]. Among these drugs, Zoledronic acid (ZDRA) has been reported to be one of the more effective BPN [13]. ZDRA is a third generation of nitrogen containing bisphosphonate, which is normally delivered intravenously. It has been shown that ZDRA has great affinity to hydroxyapatite crystals [13], inhibit the function of osteoclasts and is quite effective in treating OPR and other bone disorders [14]. For many years several direct pulp capping materials have been introduced that aim to achieve favorable pulpal healing by inducing mineralized tissue repair [15]. Studies have shown that mineral trioxide aggregate (MTA) has become the gold standard for pulp capping [2,4,16,17]. MTA has biocompatibility properties [4,16-18] and possesses antimicrobial activity [19,20], has good radiopacity, a high pH and low solubility [20]. However, MTA has a long setting time and presents some handling difficulties in a clinical setting [21].

More recently, a tricalcium silicate-based cement i.e., Biodentine (BDT; Septodont St-Maur-des-Fossés, France) has been recommended as a pulp capping material [22]. BDT is easy to manipulate, has a short setting time (approximately 12 min) and offers improved physical and chemical properties [23]. BDT is bioactive [24], exhibits excellent sealing properties [25], is biocompatible [26-28] and displays effective antimicrobial activity [29]. In direct contact with vital pulpal tissues BDT induces the synthesis of reparative dentin by secreting Nestin (a specific marker for human odontoblasts) as well as dentin sialoproteins and osteoproteins [30,31] and also by modulating the function of the growth factor TGF-β1 [32,33].

In a pilot study (Zmener et al. 2021; unpublished data) pulp capping with BDT was performed on mandibular first molars of five osteoporotic rats. After 40 days none of the animals showed signs of reparative hard tissue formation.

The purpose of the present study was to analyze the healing of pulp exposures in osteoporotic rats that were capped with BDT and pretreated with systemic administration of ZDRA. The null hypothesis postulated that there are no differences in direct pulp capping results between osteoporotic rats that are systemically treated with ZDRA and the control group that received no ZDRA.

Material and Methods

Ethical considerations

The protocol of for this study received approval from the Institutional Research Ethics Committee of the Argentine Dental Association (Protocol No. 0322/2022-AOA).

Animals and surgical procedure

This study was conducted on 15 (n=15) female white Wistar rats that had intact healthy teeth and a weight of approximately 300g. The husbandry and management of the animals met the requirements of ISO 10993–2 (2018) [34]. The operative procedures were in accordance with the ANSI/ADA Specification No. 41, 2015 [35] and with the National Research Council´s guide for the care and use of laboratory animals. Every effort was made to minimize animal discomfort and limit the total number of animals for the experiment.

The animals were maintained in cages under controlled temperature (25 ± 2°C) with 12h light/dark cycles and access to food and water ad libitum. Under strict aseptic conditions, 14 rats were induced to osteoporosis through bilateral ovariectomy. The surgery was performed by an external veterinary surgeon in accordance with the procedures described by Lasota and Danowska-Clonowska [36]. After 5 postoperative days the animals were injected for 4 days with a dose of 1mg/Kg of methyl prednisolone hemi succinate (MPH). Successful ovariectomy was verified 2 weeks after surgery by confirming cessation of the regular estrus cycle, decreased concentrations of circulating estradiol and serum progesterone. Decreased levels of the luteinizing hormone (LH) and increase in body weight were also verified. Six weeks after ovariectomy the rats were randomly assigned to two experimental groups of seven (n=7) animals each. In Group 1 the animals were treated with an intravenous dose of 100 mg/kg of ZDRA (RICHET®; Richet Laboratories S.A. Buenos Aires, Argentina), while in Group 2 (the control group) the animals received the same volume of sterile saline. On day 20, the animals received a second identical dose of ZDRA (Group 1) or sterile saline (Group 2). The remaining single healthy rat was not subjected to any treatment and the intact mandibular first molars were used as baseline controls.

Pulp Capping

One week after the second dose of ZDRA administration the animals were anesthetized through intraperitoneal administration

of 14 mg Kg/body weight of ketamine chloride and 10 mg/Kg acepromazine. They were continuously monitored to maintain complete general anesthesia during the operative procedures. They were placed on an operating board in a dorsal position and held in this position by the maxillary incisors. The right and left first mandibular molars were isolated with rubber dam with the aid of a custom-made miniature clamp and scrubbed with 10% Povidone – iodide solution (Phoenix SAIC, Buenos Aires, Argentina). Under copious irrigation with saline, Class I cavities were prepared using a high-speed hand piece in the right and left first molars using a sterile #1/2 round carbide bur (Mani, Inc, Japan). The cavities were prepared under 2.5x magnification to a depth of approximately half of the size of the bur head without exposing the pulp. A new bur was used for every animal. The pulps were then exposed by pressing a sharp sterile probe on the pulpal floor. Pulpal bleeding was controlled by light pressure with wet sterile saline cottons. Upon hemostasis the cavities were rinsed with saline, lightly dried with cotton pellets, and capped with Biodentine (BDT), which was prepared according to the manufacturer´s recommendations and applied directly on the exposed pulps. Following setting the cavities were etched with 38% phosphoric acid for 15s (Etch-Rite, Pulpdent, Watertown, MA, USA) and copiously rinsed, followed by a dentin bonding agent (Single Bond Universal, 3M, Oral Care, St. Paul, MN USA) and a composite resin (Filtek XT, 3M Oral Care). Light curing was accomplished with a Blue Phase C5 curing unit (Ivoclar Vivadent, Schaan, Liechtenstein) with a light output of 750 mW/cm^2.

Euthanasia and Sample Preparation

Thirty-eight days after pulp capping the animals were euthanized with an anesthetic overdose. After vascular perfusion with saline plus heparin and 10% neutral buffered formalin the mandibles were dissected and trimmed into block sections each containing the pulp capped first molars. They were then postfixed in 10% neutral buffered formalin for 72h, decalcified in 20% formic acid plus 8% sodium citrate following which they were rinsed in running tap water for 12h. After dehydration in ascending concentrations of alcohol, the specimens were cleared in xylene and embedded in paraffin. Sagittal serial sections of approximately 6μm thick were cut through the pulps and stained with hematoxylin and eosin.

Evaluation

The histologic sections were observed and photographed using a light microscope equipped with a Sony Cyber-shot DSC-W180 digital camera (Sony Corp, Tokyo, Japan). The images were transferred to a computer and evaluated blind by a pathologist (OZ) using Image J 1.38x Image Analysis Software (National Institutes of Health, Bethesda, MD). Histomorphometric analysis was used to determine the number of inflammatory cells along with a subsequent arithmetic mean for each specimen. The presence of inflammatory cells, fibrous tissue or necrosis, presence of reparative hard tissue formation and the morphological changes of the odontoblastic cell layer were determined by using criteria and a grading system that were modified from Al-Shama et al. [37]. A 1 to 4 scoring system, 1 being the best result and 4 the worst was used (Table 1). The results were statistically analyzed by the Fisher´s exact test using the SPSS Version 17.0 (SSPS Inc, Chicago, IL). The significance level was set at P<0.05.

Results

The two baseline mandibular first molars exhibited normal healthy pulp morphology. Of the 28 pulp capped molars (14 per group), twelve were suitable for histological examination in Group 1, while eleven were examined in Group 2. The remaining five molars were excluded from the study due to coronal fracture (two specimens) and technical errors (three specimens) during laboratory processing. The results of the histological evaluation are presented in Table 2. In Group 1 no inflammation or necrosis was observed and all samples scored a 3 for hard tissue formation. In nine a small amount of hard tissue deposition was noted at the location of pulp exposure consisting of an tubular structure without cell inclusions (Figure 1A). Deeper pulp tissue revealed the presence of dilated blood vessels (Figure 1B). In three teeth the hard tissue formation was located away from the exposure site (Figure 1C). In 4 specimens a continuous thin fibrous tissue layer was seen at the location of pulp exposure while in the remaining teeth this was interrupted. In almost all specimens the pulpal walls were lined with a regular well preserved odontoblast cell layer (Figure 1C and D).

Table 1: Scoring System Used for Histologic Evaluation.

Pulp inflammation 1:	1: Absent
	2: Mild, (<30 inflammatory cells)
	3: Moderate, (30 – 50 inflammatory cells)
	4: Severe, (>50 inflammatory cells)
Hard tissue formation	1: Complete (Total closure of the exposed area without Invading the remaining pulp, (defined as dentin bridge)
	2: Partial (extended more than the half extent of the exposed pulp area but without completely closing it)
	3: Minimal (less than the half extent of the exposed area)
	4: Absent
Fibrous tissue at the exposed area	1: Continuous
	2: Irregular
	3: Interrupted
	4: Absent
Odontoblastic cell layer	1: Regular (Presence of complete odontoblast cell layer)
	2: Irregular (Presence of empty spaces along the odontoblast cell layer)
	3: Atrophy of odontoblast cell layer
	4: Absent
Necrosis	1: Absent
	2: Present at the area under de capping material
	3: Present at the half of the coronal pulp tissue
	4. Present along the full coronal pulp tissue

In Group 2, the specimens showed neither necrosis nor reparative hard tissue formation at the pulp exposure site or elsewhere. In the area of pulp exposure an irregular fibrous tissue band was present in 4 specimens (Figure 2A and B). In 2 cases the fibrous band was continuous while in the remaining specimens the fibrous band was interrupted or absent. Dentin chips were also seen in most of the specimens of this group. Two teeth had a few inflammatory

Table 2: Overall Histologic Results for Experimental Groups.

	Group 1 (n= 12) Score				Group 2 (n= 12) Score			
	1	2	3	4	1	2	3	4
Pulp inflammation	12	0	0	0	10	2	0	0
Hard tissue formation	0	0	12	0	0	0	0	0
Odontoblast cell layer	11	1	0	0	0	0	8	3
Fibrous tissue at the exposure site	4	5	2	1	2	4	3	2
Necrosis	12	0	0	0	11	0	0	0

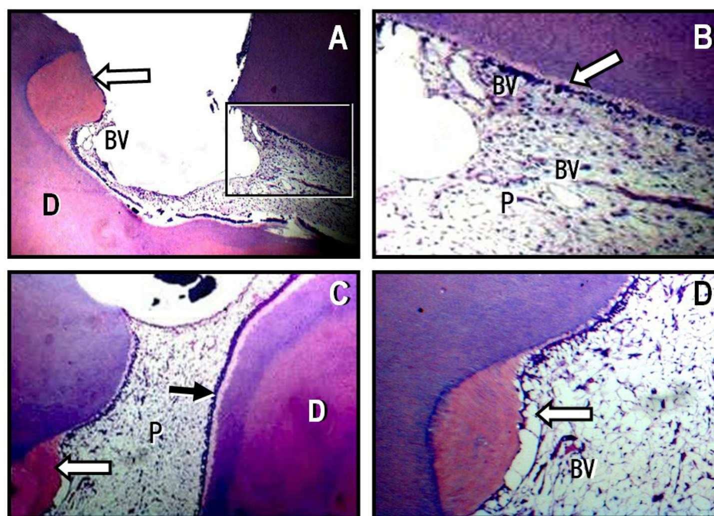

Figure 1: A to D: Microphotographs of two representative specimens of Group 1. A: Overview of the pulp exposure area showing a focal hard tissue formation (arrow) and vital pulp tissue. BV: Blood vessels. D: Dentin (Hematoxylin and eosin, original magnification X100). B: Higher magnification of the outlined square area in A. Note the presence of a continuous regular odontoblast cell layer lining the dentine wall (arrow). BV: Blood vessels. P: Pulp (Hematoxylin and eosin, original magnification X400). C: Overview of the pulp exposure area. The pulp is vital (P). Note the continuous odontoblast cell layer lining the dentin wall (black arrow). The white arrow indicates the presence of a hard tissue formation located apically from the area of pulp exposure. D: Dentin (Hematoxylin and eosin, original magnification X100). D: Higher magnification of the area in C showing the deep hard tissue formation lined by an atrophic odontoblast cell layer. BV: Blood vessels (Hematoxylin and eosin, original magnification X400).

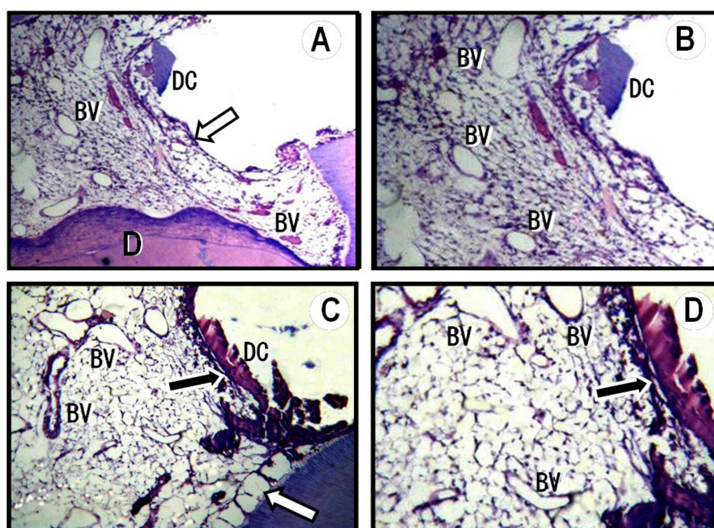

Figure 2: A to D: Microphotographs of two representative specimens of Group 2. A: Overview of the pulp exposure area showing a thin irregular fibrous tissue band (arrow) and a vital pulp with fibro reticular structure. BV: Blood vessels. P: Pulp. D: Dentin (Hematoxylin and eosin, original magnification X100). B: Higher magnification of A in which the fibro reticular pulp structure and many dilated blood vessels (BV) can be seen. DC: Dentine chip (Hematoxylin and eosin, original magnification X400). C: Overview of the pulp exposure area showing a wide blood vessel (BV) and a continuous fibrous tissue band (black arrow). Note the atrophic odontoblast cell layer (white arrow). DC: Dentine chip (Hematoxylin and eosin, original magnification X100). D: Higher magnification of C showing a reticular pulp structure, the continuity of the fibrous tissue band (arrow) and the presence of many blood vessels (BV) (Hematoxylin and eosin, original magnification X400).

cells (score 2). Atrophy of the odontoblast cell layer lining the dentin walls could be also observed in 8 specimens (Figure 2C). This was absent in the remaining 3 specimens. Although in this group most of the specimens revealed an atrophic odontoblastic cell layer lining the dentin walls, the structure of pulpal tissues was preserved and had a reticular fibrous-like appearance with dilated blood vessels (Figure 2D). The statistical analysis showed that Group 1 scored significantly better (P<0,05) compared to Group 2 in terms of inflammation, hard tissue formation, fibrous tissue at the exposure site and odontoblast cell layer organization. Therefore, the null hypothesis was rejected.

Discussion

In Endodontics it is important to ask the question in what way healing of pulpal tissue can negatively be affected by osteoporosis and what outcome of pulp capping therapy is to be expected in osteoporotic patients that are treated either with or without anti-resorptive agents. Different formulations of BPN are currently being used for the treatment of osteoporotic patients. BPN decreases hard tissue resorption by inducing hard tissue forming cell differentiation [12]. In the present study we used osteoporotic rats that were treated with ZDRA, a BPN that has been shown effective in the treatment of osteoporotic disorders [13,14]. The rat model is frequently used for direct pulp capping experiments since pulp healing has similar features in humans [38]. Al Shama et al. [37] reported complete hard tissue bridging with slight persistent inflammation 28 days postoperatively after pulp capping using a combination of bone morphogenetic protein-2 and transforming grow factor ß-1 as the capping agent in osteoporotic rats. In the current study, Group 2 was used as the control group since this group was not treated with zoledronic acid. The results of the pilot experiment (unpublished data) demonstrated that osteoporotic rats without bisphosphonate treatment did not generate calcified tissue. Furthermore, the literature is replete with articles showing pulp repair with dentin bridges in healthy animals [1-6]. BDT was chosen as a pulp capping material because of its biocompatibility [28], its ability to induce pulp healing [22,30,31] and its specific modulating function of the growth factor TGF-*ß*1 [32,33]. The absence of inflammation in all specimens in Group 1 and nine specimens of Group 2 may be attributed to the use of intact sound teeth, to a rigorous controlled operative protocol and to the inherent properties of BDT [24,25,29,39]. The small amounts of hard tissue that were observed in specimens of Group 1 could be interpreted as an initial healing attempt, which subsequently stalled, which demonstrates that the administration of ZDRA did not contribute to establishing a conducive environment for the formation of a hard bridge. The major difference in pulp reaction between both groups was the total absence of hard tissue formation in the animals that did not receive BPN. The results reported here are similar to those of Al-Shama et al. [37], who demonstrate that osteoporotic rats presented with pulp healing deficiencies in which the number of mesenchymal stem cells decreased significantly. One hypothesis is that this phenomenon could be the consequence of the reduced expression of bone morphogenetic proteins and the reduction of bone-progenitor cells, which is a common finding in osteoporotic disease [40]. Bone morphogenetic proteins are associated with proliferation and differentiation of dental pulp stem cells and the induction and formation of hard tissue repair [41].

Conclusions

Within the limitations of this study, it can be concluded that the administration of ZDRA did not appear to be effective in creating an environment that was conducive for pulp healing and the formation of a dentin bridge in osteoporotic rats. This knowledge should be taken into consideration when clinicians are confronted with a clinical case in which an exposed pulp needs treatment that deviates from a pulp exposure in a normal healthy patient. Further investigations need to confirm that the outcome of pulp capping in osteoporotic patients that are treated with BPN may be compromised.

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