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PULPOTOMY IN HUMAN DECIDUOUS TEETH AND BONE MORPHOGENETIC PROTEIN (rhBMP-2)

Pulpotomia em dentes decíduos humanos e proteína morfogenética do osso (rh BMP-2)

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Abstract

OBJECTIVES: Recent studies of pulpotomy and direct pulp capping using bone morphogenetic protein (BMP) in the teeth of animals have indicated a role for BMP in the induction and biological production of dentin. The aim of this study was to observe painful reactions and signs of clinical and radiographic pathological alterations in human deciduous teeth, as well as to histologically examine pulp tissue after the use of recombinant human BMP-2 in a collagen scaffold. **MATERIAL AND METHOD**: Five deciduous teeth for which pulpotomy had been indicated were studied. rhBMP-2 was placed in the pulp chamber of the teeth, and they were then filled using composite resin. In two teeth that exfoliated, histological examinations were performed. **RESULTS**: After twelve months, we observed 100% clinical and radiographic success, with no detectable abnormalities. On the histological sections, areas of inflammation, pulp necrosis and internal reabsorption, as well as the formation of tissue resembling osteodentin in the radicular portion, were observed. **CONCLUSIONS**: It could be concluded that the absence of symptomatology and clinical and radiographic alterations suggests that rhBMP-2 is a material with inductive properties that should be further investigated for use as an alternative to pulpotomy treatment.

Keywords: Bone morphogenetic proteins; Dentinogenesis; Cell differentiation; Pulpotomy; Biocompatible materials.

Resumo

OBJETIVOS*: A proposta deste estudo foi observar as reações álgicas, sinais de alterações patológicas (clínica e radiográfica) e examinar histologicamente o tecido pulpar de molares decíduos humanos, após o uso de BMP-2 recombinante humano em arcabouço de colágeno.* ***MATERIAL E MÉTODO****: Foram utilizados cinco molares decíduos de crianças entre oito e dez anos, com indicação de pulpotomia. Após execução da técnica, na câmara foi acomodada a rhBMP-2 em colágeno e o dente restaurado com resina composta. Estes pacientes foram acompanhados durante doze meses, dois dentes que esfoliaram foram realizados exames histológicos (H.E.).* ***RESULTADOS****: Após este período observou-se sucesso clínico e radiográfico de 100%, pois nenhuma anormalidade foi detectada. Foram observadas nos cortes histológicos, áreas de inflamação, necrose pulpar e reabsorção interna, também formação de tecido semelhante a osteodentina.* ***CONCLUSÕES****: Pode-se concluir que as ausências de sintomas e de alterações clínicas e radiográficas sugerem que as rhBMP-2 em arcabouço de colágeno são materiais com propriedades indutivas que devem ser melhor pesquisadas para que se tornem alternativa para as pulpotomias.*

Palavras-chave*: Proteínas morfogenéticas ósseas; Dentinogênese; Diferenciação celular; Pulpotomia; Materias biocomatíveis.*

INTRODUCTION

One of the objectives of science is to acquire knowledge for developing new technologies, especially in relation to materials that favor the recovery of pulp tissue that has been attacked and exposed due to trauma or caries. The literature clearly shows this pathway, with the initial utilization of materials that partially resolved the clinical problems, although with subsequent demonstration from histological studies that the pulp had suffered severe attack that could range from the formation of a surface layer of hardened tissue to the mummification of all of the pulp tissue, with the use of materials based on formaldehyde, such as tricresolformalin and formocresol. After this came the conducting materials, for example zinc oxide and eugenol, ferric sulfate and glutaraldehyde, which form a protective barrier over the pulp, in an attempt to furnish conditions for recovering the remainder of the pulp. Frequently, when this strategy is taken to have been successful, large areas of inflammation and degeneration have been shown histologically.

There has been a tendency towards investigating materials or biomaterials for returning to true pulp health via stimulation and formation of biological tissue. The first material with this characteristic was the calcium hydroxide, followed by emergence of others such as: Guedes-Pinto paste, hydroxyapatite, tetracalcium phosphate, Ca-BGP (calcium beta-glycerophosphate), MTA (mineral trioxide aggregate) and, more recently, biomaterials such as proteins for inducing cell differentiation: the bone morphogenetic proteins (BMPs) (1).

Nakagawa et al. (2) exposed human permanent tooth pulp and capped it using autogenous dentin fragments. It was observed that on the fifth day there were inflammatory cells surrounding the dentin fragments. After two weeks, crystalline structures containing calcium and phosphorus ions in their composition were found in the collagen fibrils. By the twenty-fourth day, these crystalline structures were tending to increase in size and fuse to form mineralized tissue. These findings demonstrated the existence, in the dentin fragments, of indutive factors for formation of mineralized tissue. Tziafas et al. (3) demonstrated that the bioactive components of the dentin matrix were capable of inducing cell polarization and secretion of tubular matrix, even in locations distant from the odontoblast layer, in dog dental pulp.

Okamoto et al. (4) demonstrated in subcutaneous tissue of rats that the use of dentin matrix induced formation of bone similar tissue, with the presence of bone trabeculae, cells with the

characteristics of osteoblasts and conjunctive tissue with plentiful vascularization. Likewise, some decades earlier, Urist (5) implanted demineralized bone matrix into the muscle tissue of a rabbit's leg and observed four weeks later the formation of ectopic bone. He concluded that the bone matrix contained a protein factor that was capable of self-induction, which he called *bone morphogenetic protein* (BMP). Since then, various researchers have worked on the isolation and cloning of this factor, which in reality is a combination of several inductive factors. There is a series of bone morphogenetic proteins, divided into groups and classified according to the amino acid sequences, as reported in some studies (6-11).

Around ten years ago, studies on BMPs directed towards dentistry appeared in the literature. Research has been increasing year by year, thus confirming the trend towards using tissue engineering in dental interventions. Among others, authors like Murukawa et al. (10) have worked with recombinant human BMP-2. The first of these authors implanted this protein in dental *alveoli* of rats and the others in the alveolar crests of dogs and monkeys, respectively.

It was observed that rhBMP-2 were able to stimulate, proliferate and differentiate the mesenchymal cells in osteoblasts in the alveoli, thereby preserving the bone volume and density, which could assist in performing dental implants.

BMP has also been utilized in raising the maxillary sinus, in order to increase the bone area for receiving implants. The utilization of BMPs at the time of or just after placement of the dental implant increases stability and decreases the time required for bone integration (12).

There has been research on periodontal diseases with encouraging results regarding formation of bone tissue and periodontal attachment because of the inductive effects of the bone morphogenetic proteins (13). In endodontics, experimental animal studies have shown the inductive capacity of BMPs in formation of osteodentin, or even dentin, as a biological protection for the pulp tissue (7-9,14-17).

The purpose of the present study was to observe painful reactions and clinical and radiographic signs of pathological alterations in the pulp tissue of human deciduous molars that had undergone pulpotomy and received recombinant human bone morphogenetic protein-2 (rhBMP-2) in a collagen scaffold.

CLINICAL CASES

The experiment was submitted to and granted by the Research Ethics Committee of the Universidade Metropolitana de Santos, Santos, São Paulo, Brazil.

Four healthy children aged between eight and nine years old took part in the study. These children presented five deciduous molars with lesions caused by deep decay. They came from the outpatient service of the Department Pediatric Dentistry of the School of Dentistry of Metropolitan University of Santos, with indication for pulpotomy. This was confirmed by clinical and radiographic evaluation. The pulpotomies were performed and, following this, rhBMP-2 (produced in *E. coli* at a concentration of 1 mg/ml) in a collagen scaffold (Bionnovation Produtos Biomédicos S/A – Baurú/SP, Brazil) was emplaced as a coating material.

The scaffold and the rhBMP-2 did not contain any medications or antimicrobial substances and could theoretically be considered to be an easily contaminated medium. For the protein and scaffold not to be subjected to influence from the filling material, the following protocol was utilized:

After periapical radiograph and application of the indicated anesthesia, absolute isolation was undertaken, with antisepsis using 1% chlorhexidine digluconate in order to keep the field free from contamination.

The caries were removed, so as not to leave remains of decayed tissue on the dentin walls. Opening of the pulp chamber was performed by removing the whole top of the chamber, using sterilized spherical drill bits. Using a sterilized sharpened curette, the coronal pulp was fully amputated, thus leaving to view only the pulp stumps. The coronal pulp chamber was then disinfected using cotton wool steeped in 1% chlorhexidine digluconate; pads of sterilized cotton wool were placed on the entrances to the canals using light pressure to aid in hemostasis.

Following hemostasis the rhBMP-2 with the collagen scaffold was lodged in the chamber and on the pulp stumps. On top of the rhBMP-2, a gutta-percha base was fitted in order to isolate it from possible effects due to the filling material. After placement of the gutta-percha, the cavity was acid conditioned during ten seconds, followed by washing and drying, application of the dentin adhesive, filling using composite resin and final radiograph.

These patients were clinically and radiographically followed up periodically over a one-year period. After this period, two of the teeth had exfoliated and fixed in 10% formol. After a decalcification process in 10% formic acid had been completed, semi-serial longitudinal sections of five micrometers in thickness were obtained. The slides were stained using hematoxylin-eosin (HE).

After clinical and radiographic follow-up over a one-year period, none of the teeth presented any indication of pathological alteration or painful symptomatology.

MICROSCOPIC FINDINGS

Microscopic examination showed suppurative foci, foreign body giant cells, external and internal reabsorption and lacunae with multinucleated giant cells (Figures 1, 2 and 3).

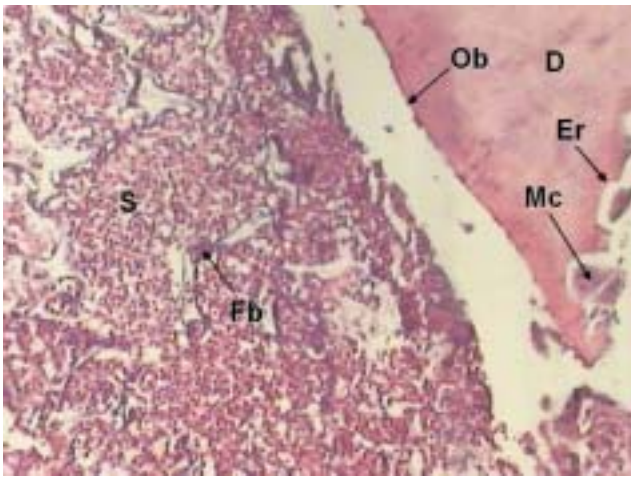

FIGURE 1 - In the center, suppurative area (S); giant cells with foreign body (Fb); odontoblasts (Ob); dentin (D); external reabsorption (Er) with giant multinucleated cells – HE 100x

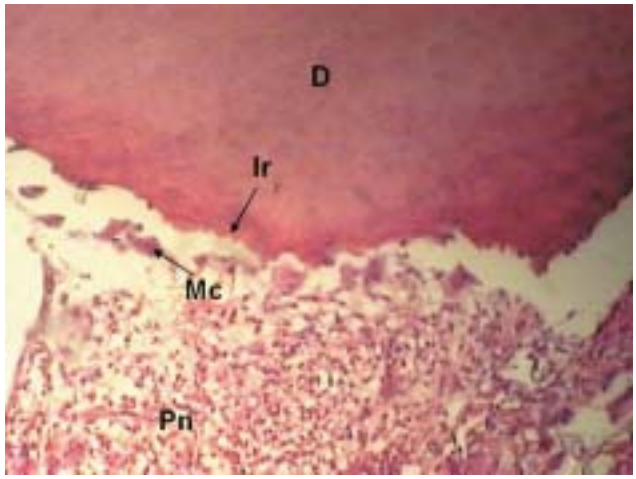

FIGURE 2 - Dentin (D), internal reabsorption (Ir) plus multinucleated cells (Mc); inflamed pulp tissue with polymorphonuclear cells (Pn) – 100x

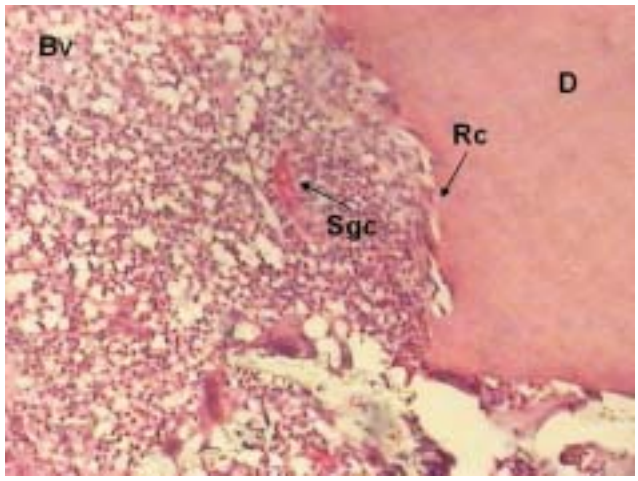

FIGURE 3 - Dentin (D); reabsorption with cells (Rc); suppurative tissue plus giant cells and blood vessel – HE 100x

In one tooth the coronal pulp chamber was broad and contained amorphous basophilic material that was similar to osteoid or dentin matrix, sometimes surrounded by foreign body giant cells and slight to moderate inflammatory infiltrate with neutrophils. On the periphery there was normal pulp tissue in contact with the dentin (Figures 4, 5 and 6).

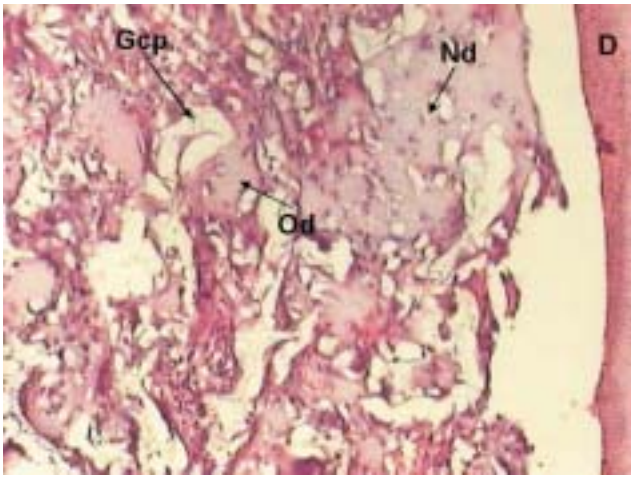

FIGURE 4 - Dentin (D); mass with cells inside it suggesting new formation of dentin (Nd); eosinophil tissue suggesting osteoid dentin; giant cells displaying phagocytic activity towards foreign body; and giant cells at the base of the larger mass – HE 100x

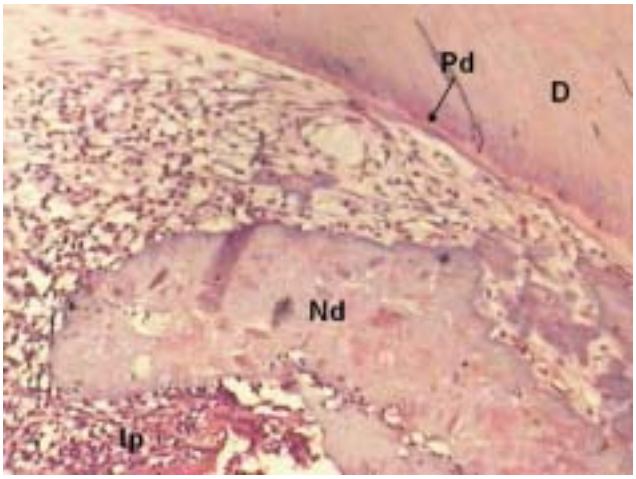

FIGURE 5 - Dentin (D); pre-dentin (Pd); island of tissue resembling new formation of dentin (Nd); acute inflammatory process at the lower margin (Ip); it is noteworthy that there are no giant cells for phagocytic activity – HE 100x

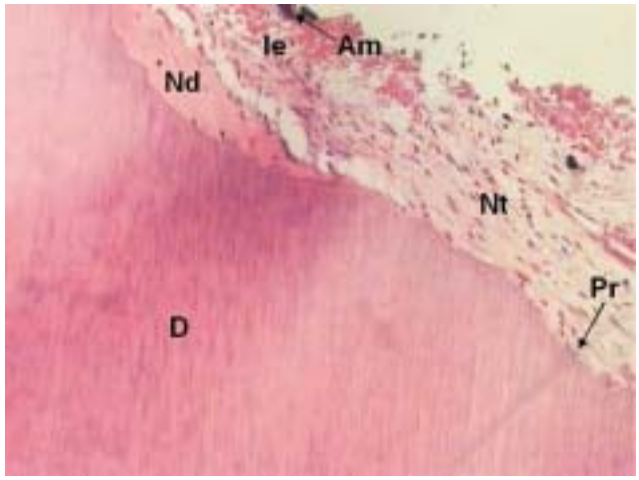

FIGURE 6 - Dentin (D); physiological reabsorption (Pr); mixed inflammatory exsudate (Ie), normal pulp tissue (Nt); amorphous material (Am); new formation of dentin suggesting repair (Nd) – HE 100x

DISCUSSION

In 1990, when the different groups of BMPs had not yet been isolated, Nakashima (1) utilized BMP extracted from animal bone material, on the exposed pulp of dog teeth. Four weeks later, he observed the formation of osteodentin in various parts of the display. In the eighth postoperative week, odontoblasts were observed, forming tubular dentin close to the osteodentin. These results were perhaps obtained because of the inductive effect of a veritable "pool" of bone morphogenetic protein.

The proteins most studied in pulp tissue have been BMP-2, BMP-4 and BMP-7 (OP-1). This can be seen from the studies of Rutherfor et al. (8, 14, 18), Jepsen et al. (7) Six et al. (11) who utilized rhBMP-7 (rhOP-1) in a collagen scaffold, and Nakashima (6, 15, 17) Ren et al. (9) and Iohara et al. (16), who utilized BMP-2 and BMP-4.

A sequence can be built up for the healing over of the pulp, which can be summarized as follows:

Cells similar to fibroblasts migrate from the lower pulp tissue to the amputation zone (free from contamination), where they proliferate. Following this, there is formation of inactive matrix or utilization of the scaffold itself, for the stem and undifferentiated mesenchymal cells to adhere to. BMP-2 -4 and -7 induce the differentiation of the adhered cells into odontoblasts that, in their turn, take part in the production and mineralization of the dentin matrix.

In vivo experiments by Rutherford et al. (14, 18), using rhBMP-7 in dental pulp of monkeys, demonstrated that in the first month following the intervention healing was characterized by capillaries and cells surrounded by mineralized extracellular amorphous matrix. By the sixth month, there was a wide band of mineralized dentin with non-tubular regions and flattened cells, while in other areas pre-dentin was seen, associated with dentin of tubular appearance. Isolated lacunae containing vestiges of blood and some isolated cells appeared, irregularly distributed by means of the repairing dentin.

According to these authors, the formation of the mineralized tissue is dose dependent. Six et al. (11), using rat pulps concluded that on the eighth day a moderate initial inflammation was observed, with increased numbers of undifferentiated cells and formation of matrix close to the exposure.

By the twenty-eighth day the inflammatory process had regressed and mineralized tissue was sealing off the exposed pulp, while there was dentinogenesis activity in the root portion that demonstrated a tendency towards obliteration of the canal with recently formed homogeneous and tubular dentin.

In the studies in dogs using rhBMP-2 and -4 in a scaffold of collagen type I 6,9,15, histological evaluation performed close to the seventieth day showed that part of the pulp stump presented mineralized tissue that was similar to osteodentin, with the presence of capillaries and osteodentin cells with circular or oval nuclei in irregular lacunae.

Below the osteodentin bridge, there was presence of irregular tubular dentin and some odontoblasts. On another site, fibrous tissue that was still unmineralized was observed, with fusiform cells producing extracellular matrix.

The pulpotomy procedures performed on humans in the present study produced a small sample of two human deciduous teeth treated with rhBMP-2 in a collagen scaffold, between six and eight months later. Although the small sample size makes it impossible to come to effective conclusions, the histological sections showed areas of inflammation, pulp necrosis and internal reabsorption. They also showed matrices and islands of calcified tissue resembling osteodentin and reactional dentin in certain regions (Figures 4, 5, 6).

Despite the possible induction and formation of mineralized tissue, the histological picture presented negative characteristics of chronic inflammation, pulp necrosis and reabsorption. This was over a period of time in which aspects of the recovery of the pulp tissue should have been observed. These conditions may have been caused by two relevant factors.

The first relates to the pulp components, especially the numbers, kind and stages of the cells since, for induction to take place, there is a need to recruit a large number of cells (stem cells and undifferentiated mesenchymal cells and fibroblasts), that are capable of differentiation.

It is known that, in the life cycle of the tooth, the structural elements of the pulp and the numbers of cells differ in the various phases, as was demonstrated by Bhussry (19) in rat dental pulp, in which with increasing age there was a decrease in the cell population. The developing mesenchymal cells of the pulp differentiate into fibroblasts, and it is observed that in mature pulp they appear in decreased numbers, sparsely distributed. Araujo (20) researching on human deciduous teeth in different phases of the cycle, extracted because of orthodontic necessity, concluded that the structural alterations observed in the pulp tissue at three different phases of root reabsorption could not be considered to be degenerative. However, histometrically the number of fibroblasts was significantly lower at the end of the root reabsorption than in the initial and middle phases of the reabsorption.

With regard to the collagen fibers, there was a significantly greater number at the end of rhyzolysis. Likewise, the number of vascular structures was lower and, morphologically, there was an increase in the caliber of these vessels by the end of the reabsorption. In our study, the deciduous molars in which the pulpotomy procedures were performed and rhBMP-2 in a collagen scaffold was utilized were in the final phase of rhyzolysis. At this time, the number of cells was lower, and this could provide a partial explanation for the lack of cell differentiation.

The second factor is related to the effectiveness of the dentinogenesis induction capacity of the BMPs under different physiological or pathological conditions of the dental pulp. The experiments for evaluating the inductive effect of various types of BMPs were done on healthy pulp that was exposed or underwent pulpotomy by surgical means, under ideal conditions (1, 6-9, 11, 14, 15, 18, 21-23). We found only one study in the literature that simulated inflammation: Rutherford (8) intentionally provoked moderate and reversible inflammation using an aqueous solution of *Salmonella typhimurium* lipopolysaccharide, prior to utilizing rhBMP-7 on the partially amputated pulp of ferret canine teeth. These authors concluded that rhBMP-7 failed to induce reparative dentin in teeth with reversible inflammation. In the present study, despite all the care with antisepsis and isolation using a rubber dam, the pulpotomy procedures that we performed were on teeth with lesions due to deep decay, and therefore the pulp presented some degree of inflammation.

By taking together these considerations with the clinical/radiographic follow-up and the histological results from the human deciduous teeth treated with rhBMP-2, we can conclude that there is no doubt that the way forward is to seek appropriate biomaterials. Such agents would be able to increase the healing potential of the pulp conjunctive tissue through the induction of large quantities of dentin or osteodentin on the exposed pulp. Such agents would isolate and protect the pulp from micro-infiltration and possible future infection. New research using cell and molecular biology techniques should be undertaken with the aim of clarifying the involvement of biological markers contained in the expressed DNA segments and the diverse and complex mechanisms that govern the induction, repair, maturation and degeneration of cells and tissues.

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