Bulgarian Journal of Veterinary Medicine (2007), **10**, No 1, 35–43

TISSUE RESPONSE OF DENTAL PULP IN DOGS FOLLOWING DIRECT CAPPING WITH POTASSIUM NITRATE IN POLYCARBOXYLATE CEMENT

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Summary

Borissov, I., S. Tsanova & D. Sivrev, 2007. Tissue response of dental pulp in dogs following direct capping with potassium nitrate in polycarboxylate cement. *Bulg. J. Vet. Med.*, **10**, No 1, 35–43.

In 6 dogs, experiments were performed to determine the tissue reaction of the dental pulp at the 7th, 30th and 60th days after direct contact of the pulp with the obturation material through a microcommunication orifice with diameter up to 1 mm, formed in the fundic part of an experimental cavity. Capping of 36 mandibular premolars with traumatically exposed pulp was carried out. On the exposed pulp, polycarboxylate cement with 5% potassium nitrate was applied on 18 teeth (experimental) and a preparation containing calcium hydroxide (Calxyd) – on the opposite 18 (control) teeth. The histological preparations were observed by light microscopy and the dental pulp changes were determined by the Negm's scale using 3 scores: weak, medium and strong inflammatory reaction. The status of the odontoblast layer was evaluated after Qvist & Qvist and the amount of reparative dentin – after Stanley. The obtained results showed that the direct capping of traumatically exposed dental pulp with potassium nitrate in polycarboxylate cement did not cause degenerative changes in the pulp-dentin complex. The histomorphological tissue response was probably due to effect of potassium nitrate and polyacrylic acid upon the regeneration in the dental pulp protected against microbial infection. It could be supposed that the good adhesion properties of the material ensured the isolation of the exposed dental pulp from the environment, creating optimal preconditions for manifestation of its natural reparative potential.

Key words: dogs, polycarboxylate cement, potassium nitrate, pulp cap

INTRODUCTION

The mechanical traumas of teeth amount to 27% of dental pathology in dogs (Smith, 2002; Rawlinson, 2003). They are most commonly encountered in police dogs and affect mostly canine and incisive teeth (Brech & Hamel, 1997; Van Foreest & Roeters, 1998).

The vital pulp therapy, performed timely and with effective biocompatible pulp capping materials as an alternative to the routine endodontic treatment, contributes to the successful healing and protection of the vitality of fractured teeth. The favorable outcome of the treatment with polycarboxylate cement containing 5% potassium nitrate is determined, from one part, by the length of exposure time and on the other – by the biological tolerance of pulp capping materials. It is accepted that the vital pulp therapy should be car-

ried out up to the 48th hour after the traumatic pulp exposure (Niemiec, 2001; Clarke, 2001). The duration of pulp exposure correlates with bacterial contamination and the degree of dental pulp local inflammation (Tang *et al.*, 2002).

The purpose of the vital pulp therapy is the regeneration of dentin and restoration of the normal architectonics of the damaged pulp-dentin complex. For this purpose, in different clinical cases, various pulp capping materials have been utilized: calcium hydroxide-containing drugs, enzymes, adhesive systems, growth factors, mineral substances (Komnov, 1989; Kiba *et al.*, 2000; Souza *et al.*, 2001; Cardenas *et al.*, 2002; Tziafas *et al.*, 2002).

There are conflicting views about the safety of calcium hydroxide as a pulp capping agent (Cox *et al.*, 1998). It is shown (Trope *et al.*, 2002), that the pulp healed better if capped with a bonding system (IV generation) instead of $Ca(OH)_2$ followed by placement of a glass ionomer cement liner. In a primarily exposed pulp however, irreversible pulp damage could occur and loss of vitality following a total etching (Kiba *et al.*, 2000).

An alternative preclinical model of vital pulp therapy, aiming at the reconstruction of the normal tissue architecture directly on the dentin-pulp interface (Tziafas *et al.*, 2001) is the application of biocompatible and biodegradable enamel and dentin matrices (Tziafas *et al.*, 2002; Ishizaki *et al.*, 2003). The reparation process occurs on the basis of direct induction of odontoblast-like cells and formation of reparative dentin. The plastic properties of the pulp for formation of secondary dentin is mainly conditioned by proodontoblasts, originating from existing and migrating mesenchymal cells.

According to some authors (Ren *et al.*, 2000) the direct capping of exposed dental pulp with a fibrin sealant (FS) results in fewer pulp damage but FS lacks a dentin-inductive activity. Very good results about increase in the tertiary dentin after 4 and 8 weeks were obtained by Ishizaki (2003) for the histopathological dental pulp response after application of enamel-matrix derivative (EMD) as pulp capping material. The early pulp cell response, after application of a mineral trioxide aggregate (MTA) for dentin-induced dentinogenesis, showed the formation of reparative dentin via a stereotype protective mechanisms of early pulp healing (Tziafas *et al.*, 2001, 2002).

In the literature, there are limited data about the effect of KNO_3 on the pulp complex including comments on its effect upon the traumatically exposed dental pulp (Tsanova & Chokova, 1992; Tsanova, 2002; Borissov *et al.*, 2003). That is why, on the background of data about the effect of various pulp capping materials, used up to now, the pulp tissue reaction after direct pulp capping with potassium nitrate in polycarboxylate cement, a therapeutical method proposed by Hodosh *et al.* (1983) is of interest.

The purpose of the present study was to continue our observation (Borissov *et al.*, 2003) on the histomorphological pulp response in dogs after direct capping of traumatically exposed dental pulp with potassium nitrate in polycarboxylate cement.

MATERIALS AND METHODS

Experimental animals

The studies were performed in 6 mixed-breed dogs aged between 2 and 3 years and weighing 15−20 kg. Two weeks prior

to the experiment and during the study, the animals were fed and housed uniformly. The dogs were housed in individual cages with an area of 1.5 m^2 and height 1.8 m, provided with feeding and drinking bowls and cleansed daily. They received commercial dry food (20–30 g/kg) and water *ad libitum*. The studies were performed observing the requirements for human attitude towards animals.

In each dog, 6 cavities were prepared of mandibular premolars, the left 3 cavities served as control (totally 18, group A) and the right 3 cavities (totally 18, group B) were the experimental ones.

Anaesthesia

Following s.c. premedication with atropine sulfate (Atropinum sulfuricum ampoules of 1 mg/mL, Sopharma, Sofia, Bulgaria) at 0.02–0.04 mg/kg, an intravenous anaesthesia with xylazine (2% Rometar, Spofa, Prague, Czech Republic) at 2 mg/kg and ketamine (Imalgène, Rhône Mérieux, France) at 6-10 mg/kg was performed. The anaesthetic effect occurred after 15−20 min and lasted for 30−60 min.

Teeth and cavity preparation

Under both water and air cooling, 3 ×5 mm cavities of class V were prepared in buccal surfaces of experimental teeth. The cavity floor was thinned to a pulpar dentin thickness of 1.0 mm using a round sterile borer. The direct contact between the pulp and the obturation material in the fundic part of the experimental cavity was attained by creation of a microcommunication opening with a diameter up to 1 mm. The experimental cavities of each dog were washed with sterile saline, dried with sterile cotton buds, and capped with polycarboxylate cement containing 5% potassium nitrate. The control cavities were identically treated and capped with a calcium hydroxide containing product (Calxyd[1], Spofa Dental, Prague, Czech Republic) as a protective base. All cavities were hermetically closed with a temporary seal (Cavit[2], Spofa Dental, Prague, Czech Republic).

Histological studies

At post treatment days 7, 30 and 60, 6 experimental and 6 control teeth were extracted using the same xylazine-ketamine anaesthesia, for each experimental period. Immediately, with a diamond separator and water cooling, dental roots were removed and the rest of the tooth was cut into 3 mm slices and fixed in 10% neutral buffered formalin. The fixed slices were decalcinated according to the method of Volkova & Eletskii (1986) and processed by the routine histological procedure: embedding in paraffin blocks in a thermostat at 56 ^{o}C with a subsequent cooling and bucco-lingual cutting of 5-6 μm histological sections with a microtome. After deparaffination, hydratation and clearing, the sections were stained using three staining techniques: with haematoxillin/eosin (for histomorphological examination), with acid picrofuchsin according to Van Gieson (for observation of collagen fibres) and according to Mallori (for observation of reticular fibres).

After fixation and covering on standard observation glasses, the stained experimental and control histological sections were observed on an Amplival light

[1] contains two components: 1. a paste composed of zinc oxide, calcium phosphate, calcium tungstate and 1,3-butylene glycol and 2. a catalyst composed of calcium hydroxude, zinc oxide, titanium dizinc stearate and iron; both components are mixed prior to use.

[2] containing (in 100 g): zinc oxide 30.8 g, calcium sulfate 30.8 g, zinc sulfate 15.4 g, vaseline 22.6 g and menthol oil 0.4 g.

microscope at magnifications of 6.3×10; 10 ×10 and 40 ×10.

Assessment of findings

The changes in dental pulp were evaluated using the Negm scale as followed: weak, medium and considerable degree of inflammation.

The status of the odontoblast layer was evaluated according to Qvist & Qvist (1977) with the following indexes: 0 − no reduction; 1 − insignificant reduction and 2 − medium to significant reduction.

The quantity of reparative dentin was determined by the method of Stanley on a 3−degree scale: 0 − no reparative dentin; ½ − initial formation of reparative dentin and 1− presence of a significant amount of reparative dentin.

RESULTS AND DISCUSSION

In experimental traumatic pulpitis (pulpar collision) and direct capping with polycarboxylate cement containing 5% potassium nitrate, the 7-day contact with the exposed dental pulp in the experimental group resulted in the following histomorphological changes:

- medium degree of inflammation (according to Negm) with haemorrages and necroses in the region of communication, with separate dentin debris in the pulp and more profoundly, dilated blood vessels. Microabscesses were absent;
- index 2 by Qvist with reduction in the number of odontoblasts immediately in the area of communication with the pulp, areas of disorganization (1 by Qvist) adjacently to the damaged area;
- degree 0 by Stanley – reparative dentin is lacking, and the predentin strip is narrowed, being absent nearby the exposed dental pulp.

This histomorphometrical picture was not significantly different from that in the preparations of the corresponding control group of teeth, where the calcium hydroxide preparation Calxyd was used for capping of pulp. In both groups (experimental and control), the alterations prevailed after a 7-day contact of the pulp capping material and the exposed pulp (Fig. 1).

After the 30-day direct contact with the polycarboxylate cement containing 5% potassium nitrate, the following histological picture was observed:

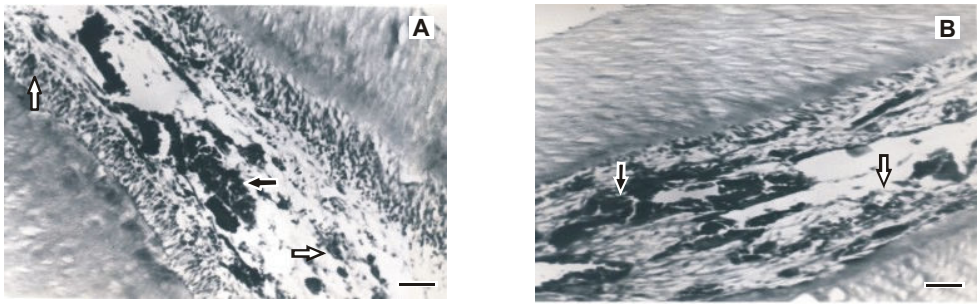

Fig. 1. Morphological changes in dental pulp in a dog 7 days following direct capping: A. Cavities, treated with polycarbocylate cement containing 5% KNO_3 (experimental) – focal haemorrhages (white arrows) and necroses (black arrows) could be seen. B. Cavities, treated with Calxyd (control) – focal haemorrhages and necroses are also present; HE, bar = 10 μm.

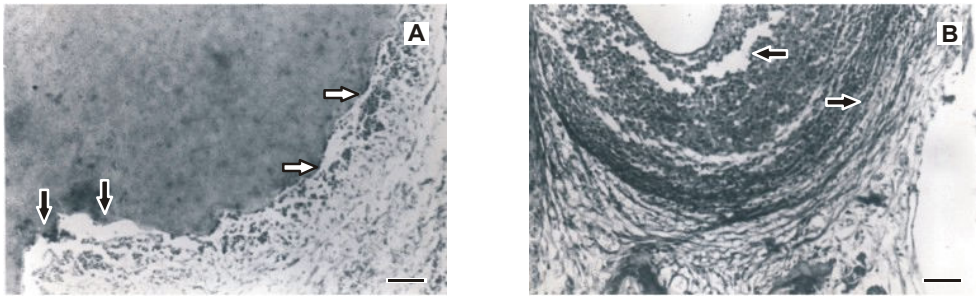

Fig. 2. Morphological changes in dental pulp in a dog 30 days following direct capping: A. Cavities, treated with polycarbocylate cement containing 5% KNO_3 (experimental) – resorbed necroses (black arrows), persisting haemorrhages and cell inflammation infiltrate (white arrows) are observed; B. Cavities, treated with Calxyd (control) – exudative inflammation (horizontal black arrows) could be seen; HE, bar = 10 μm.

- weak inflammatory reaction according to Negm – resorbed necroses, persisting haemorrhages, cellular inflammatory infiltrate containing macrophages, few leukocytes and more lymphoid cells, limiting the damaged pulp area;
- Qvist index 1 with reduction in odontoblast counts and areas of disorganization in the odontoblast layer;
- Stanley index 0 – lack of reparative dentin, but the appearance of fibroblastic elements was indicative for the beginning of reparative processes in the pulp.

In the 30-day control group of teeth, similar histomorphological changes were observed. In both groups, there was an exudative inflammation as a pulp response to preceding damages (Fig. 2).

The histological features of the material, obtained by the 60th day from the experimental group, were as followed:

- absence of pulp inflammatory reaction evaluated according to Negm. Higher fibrocyte counts with collagen fibres bundles were observed after Van Gieson staining;
- Qvist index 1 with insignificant reduction in the number of odontoblasts;
- Stanley index from 0.5 to 1 – reparative and restorative processes with local calcium deposits.

The Mallori stained preparations showed that the reticular network of the pulp was preserved and the reticular fibres were gathered mainly around the blood vessels.

In the 60-day control group, a restrictive capsule between necroses, haemorrhages and inflammatory infiltrate from one part, and the healthy pulp from the other, was observed. Also, a persisting exudative pulp inflammation with diffusely spread round cells was present (Fig. 3).

The evaluation of obtained results showed that by the 7th day after direct capping of traumatically exposed dental pulp with polycarboxylate cement containing 5% potassium nitrate, it reacted with inflammation, manifested by haemorrhages and necroses. The absence of microabscesses as well as a diffuse involvement of the pulp in the inflammation was most probably due to the antibacterial

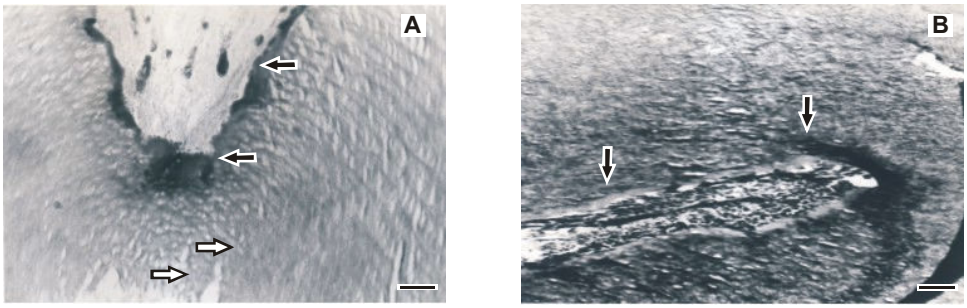

Fig. 3. Morphological changes in dental pulp in a dog 60 days following direct capping: A. Cavities, treated with polycarbocylate cement containing 5% KNO_3 (experimental) – reparative restoration processes (black arrows), increased fibrocyte amount and reduction of odontoblasts (white arrows) are present; B. Cavities, treated with Calxyd (control) – the between the healthy and exudatively inflammed dental pulp, a restrictive capsule could be seen (vertical black arrows); HE, bar = 10 μm.

effect of potassium nitrate and polyacrylic acid, as a matrix of the pulp capping material, limiting the penetration of microorganisms in profundity (Shovelton *et al.*, 1971; Tsanova & Chokova, 1992). The alterations in the normal pulp structure, located just under the capping material, were most likely due to the direct mechanical trauma following the preparation of the cavity (Watts & Paterson, 1987; Stanley & Swedlow, 1959).

Thirty days after the direct capping of the pulp with polycarboxylate cement containing 5% potassium nitrate, there were no necroses in both experimental and control groups, but only persisting haemorrhages and cellular inflammatory infiltrate composed of macrophages, a few leukocytes and more lymphoid cells, restricting the damaged pulp area. There was an exudative inflammation, as a pulp response to preceding damages. No pathological complications were observed, evidencing a good protection of the pulp.

By the 60th day, the data in experimental teeth showed that the pulp inflammation was over and the reparative restorative processes had begun.

In control teeth, the haemorrhages, necroses and inflammatory infiltrate in the area of communication were separated from the healthy pulp by a connective tissue capsule. It was composed by fibroblast elements, fibrocytes, small blood vessels and peripherally located collagen fibres. The odontoblast layer was necrotic and the persistent hyperaemia and the presence of elements of inflammation showed a chronification of pulp inflammation.

The *in vivo* comparative study of pulp capping materials polycarboxylate cement containing 5% potassium nitrate (experimental group) and the calcium hydroxide-containing preparation Calxyd (control group), applied directly on the pulp after creation of microcommunication, showed the better adhesion of the former compared to the latter. The adhesion was most probably due to the ion exchange between the dentin and the cement and the formed bond is very difficult to be broken (Walter, 1973; Tsanova, 2002).

It could be supposed that in the studied method, the polycarboxylate cement ensured a maximum hermeticity of the dentin wound (Pakh, 1987; Van Dijken, 1996) and manifested a desensitizing ef-

fect (Gillam *et al.*, 1996) and reduced to a minimum the risk of crack formation between the dentin and the obturation (Alacam *et al.*, 2000; Borissov *et al.*, 2003). Another advantage of this method could be the achievement of a very good mechanical resistance and the formation of alkaline environment, possessing a strong antiacidic, anti-inflammatory and antibacterial effect of this pulp capping material (Tsanova & Chokova, 1992).

The treatment of the pulp with 5% potassium nitrate in polycarboxylate cement applied directly on the pulp after creation of a microcommunication influenced positively the pathogenetic mechanisms of inflammation, restored the haemodynamics in the affected pulp segment, achieved a complete marginal adaptation and ensured optimal conditions for stimulation of regenerative potential. An objective criterion for that was the reparative dentin accretion, proved by histomorphology tests. This experimental method for pulp treatment probably works by improvement of microcirculation in the affected pulp segment, stimulation of the natural reparative potential of the dental pulp and provision of optimal biological conditions for the healing process (Schilder, 1967).

The comparison of obtained results with previous data of ours (Tsanova & Chokova, 1992; Tsanova, 2002; Borissov *et al.*, 2003), where a direct contact between the pulp and the capping material was absent showed that the direct contact does not result in different dental pulp reaction and that in both cases, optimal conditions for activation of the reparative process were created.

The results from the experiments allow us to propose the method of direct capping of traumatically exposed dental pulp with polycarboxylate cement containing 5% potassium nitrate as an alternative for biological treatment of pulpitis.

CONCLUSIONS

The application of polycarboxylate cement containing 5% KNO_3 for direct capping of traumatically exposed pulp, did not result in any degenerative changes in dental pulp.

The good adhesive properties of polycarboxylate cement containing 5% KNO_3 guaranteed the closure of cavity, good and lasting protection of traumatically exposed dental pulp, creating optimal conditions for expression of its natural reparative potential.

The obtained experimental data from the direct capping of traumatically exposed dental pulp with polycarboxylate cement containing 5% KNO_3 could serve as a basis for clinical investigations.

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* Author's translation

Paper received 16.06.2004; accepted for publication 23.11.2006

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