BIOCOMPATIBILITY OF DENTAL PULP CAPPING MATERIALS: A HISTOLOGICAL STUDY

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Keywords: *biocompatibility, dental pulp*

Abstract: *The aim of this article is to evaluate the biocompatibility of materials used in pulp capping procedures, by measuring the degree of inflammation induced by these products after subcutaneous implantation in rats. We used 12 animals divided in 3 study groups and 1 control group; implants with Mineral Trioxide Aggregate (MTA), Life cement and Calxyd were placed in subcutaneous tissue and histological evaluation was carried out after 7, 14 and 21 days. We obtained comparable results between MTA and Life cement, which demonstrates similar degrees of biocompatibility. Due to excellent long-term clinical results, the replacement of calcium hydroxide containing materials with MTA is not necessary. Further clinical studies are needed to evaluate the potential of MTA to induce dentin bridge formation on exposed dental pulp.*

Cuvinte cheie: *MTA, biocompatibilitate, pulpă dentară*

Rezumat: *Scopul studiului este de a evalua biocompatibilitea materialelor de coafaj, prin realizarea de implanturi subcutanate la animale de laborator. 12 animale au fost împărțite în 3 grupe de studiu și una de control, la care am efectuat implanturi cu Mineral Trioxide Aggregate (MTA), ciment Life și pastă Calxyd. Specimenele au fost recoltate la interval de 7, 14 și 21 zile, colorate cu HE și evaluate în manieră dublu-orb. Am obținut valori comparabile ale intensității reacțiilor inflamatorii în cazul MTA și a cimentului Life, ceea ce demonstrează grade similare de biocompatibilitate. Putem susține că, datorită rezultatelor excelente confirmate pe termen lung, înlocuirea materialelor cu hidroxid de calciu cu MTA nu se impune. Sunt necesare studii clinice viitoare care să evalueze potențialul MTA de a forma punți de dentină după aplicarea sa pe suprafața pulpei dentare și de a evalua rezultatele în timp.*

INTRODUCTION

Biocompatibility is a condition that must characterize all materials used in conservative dentistry, which means no risk of side effects on contact with host tissues.(1,2) In the case of pulp capping materials, this is expressed by many variables such as genotoxicity, mutagenicity, carcinogenesis, cytotoxicity, histocompatibility and antimicrobial effect.(3,4) Calcium hydroxide Ca(OH)2 was the most used material in conservative dentistry, which in contact with vital pulp tissues determines a small area of necrosis accompanied by a mild inflammatory reaction.(5) In the absence of microorganisms, reparative reactions take place and a dentin bridge is formed underneath the Ca(OH)2. Unfortunately, this material has a marked tendency to dissolve in time, leaving empty spaces, which represent opportunities for bacterial infiltration. Taking into consideration that $Ca(OH)_2$ cements induce the development of dentin bridges with tunnel-like defects, that allow bacterial penetration from the oral cavity to the pulp tissue, other materials were introduced with less marginal leakage and better isolation of the pulp chamber. Over the years, researchers used zinc oxide eugenol, glass ionomer cements, dentinal adhesive systems and, recently, a new material called "Mineral Trioxide Aggregate" (MTA). Due to its alkaline pH, it was shown to stimulate dentin bridge formation, with very good results in direct pulp capping procedures.(6)

PURPOSE

The purpose of our study was to evaluate the biocompatibility of frequently used materials in conservative treatment of vital pulp, such as MTA and two Ca(OH)2 containing products: Life cement and Calxyd paste. We measured the degree of inflammation induced after subcutaneous implantation of these materials in experimental animals.

METHODS

According to international laws, our test was conducted respecting the criteria of ISO 10.993 and ISO/Tc 194; we obtained the acceptance No. 58/2011 from Committee for Ethics of Research of the University of Medicine and Pharmacy of Tîrgu-Mureş. As bio-test, we used a species of laboratory rats and the materials included in this study were implanted in subcutaneous tissue. There were 4 groups consisting of 12 animals, 3 study groups and 1 control. Each study group received implants of sterile cotton with MTA, Life cement (Kerr) and Calxyd (Spofa Dental) respectively, and in the control group only sterile cotton pellets were implanted.

The procedure was carried out by a single operator, who used local anesthesia with 2 ml Mebumal 10%, following general rules of asepsis and antisepsis; postoperatively, the animals were not isolated and no antibiotherapy was used, in order to avoid any effect on tissue inflammatory reactions. Under the same protocol, specimens of connective tissue were obtained at 7, 14 and 21 days. All fragments were immediately introduced in formaline solution and prepared for histological evaluation. We used hematoxylin-eosin and/or PAS stains and the evaluation was carried out by one examiner, in a double-blind manner.

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Article received on 07.01.2014 and accepted for publication on 23.01.2014
ACTA MEDICA TRANSILVANICA March 2014;2(1):217-220

The degree of inflammation was evaluated in 4 stages, based on the criteria of Commission of Dental materials, instruments, equipment and techniques (1980) in the following manner:

0 – absent: width of inflammatory zone similar to control group, absence of or only a few inflammatory cells (no more than 5 cells);

1 – moderate: mild inflammatory reaction, macrophages and plasma cells (5-25 cells);

2 – intense: very strong inflammatory reaction, macrophages, plasma cells, with foci of granulocytes and lymphocites (25-100cells);

3 – severe: areas of necrosis, numerous inflammatory cells in the surrounding tissue (more than 125 cells).

Quantitative assessment of inflammatory cells was carried out in 5 separate fields of each specimen, the mean count was determined and the severity of inflammatory response was noted. Data from the experimental groups (MTA, Life cement and Calxyd) and control group was compared using Friedman statistical test for each period of time (7, 14 and 21 days). Wilcoxon complementary test was used to determined differences between study groups ($p< 0,05$).

RESULTS

The severity of tissue inflammatory response for all these dental pulp capping materials was high. The most severe reaction was noted in the case of Calxyd, followed by MTA, Life and control groups. Cellular distribution scores for the experimental and control groups and the corresponding tissue reactions are presented in table no. 1.

Table no. 1. Cellular distribution scores in all groups for each time interval

Cells	Time	7 days				14 days				21 says			
	Study group	A	B	C	D	A	B	C	D	A	B	C	D
PMN Leucocytes		3	3	3	2	2	2	1	0	1	1	1	0
Lymphocyts		3	3	2	2	2	2	1	1	1	1	0	0
Macrophages		2	1	2	1	1	2	1	0	1	0	0	0
Giant cells		2	1	1	1	1	1	0	0	0	0	0	0
Necrosis		+	+	+	+	+	+	-	-	-	-	-	-
Fibrous capsule		-	-	-	-	-	+	+	+	+	+	+	+

Overall, the inflammatory cell infiltrate decreased from day 7 to day 21 in all groups and the development of a collagen membrane around the implant increased during the study period. A fibrous capsule formation was completed in the control group by day 21. After 7 days, the Friedman test indicated a significant difference between the groups ($p< 0,05$) with Calxyd paste showing the most severe response. On the other hand, the Wilcoxon complementary test did not show significant differences between Life cement and MTA. After 14 and 21 days, the Friedman test indicated significant differences between the groups ($p< 0,05$), but with the Wilcoxon complementary test there were no differences between Calxyd – control, MTA – control and Life – control groups. The sealer groups did not show any significant differences among them (table no. 2).

Table no. 2. P values regarding the intensity of inflammatory reactions induced by different experimental materials

Group / Tim	Calxyd	MTA	Life	Control
7 days	0.275	0.317	0.163	0.011
14 days	0.502	0.448	0.316	0.038
21 days	0.319	0.229	0.173	0.014

In the following pictures, we present the histological aspects obtained from all study groups at different time intervals (figures no. 1-5).

Figure no. 1. Life cement at 14 days. Connective tissue barrier is developing around the implant, the inflammatory infiltrate is moderate. There are numerouse collagen fibres and fibroblasts which express tissue healing reactions. (H&E stain, 20X)

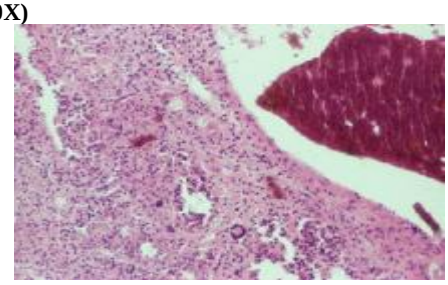


Figure no. 2. MTA at 14 days. Slight tendency to tissue healing and reduction of the inflammatory infiltrate. Connective tissue barrier was formed, there are numerous collagen fibres and a few macrophages. (H&E stain, 20X)

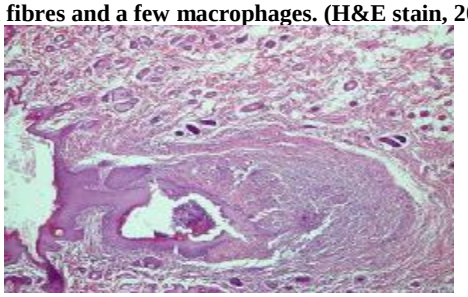


Figure no. 3. Control at 21 days.The implant placed in subcutaneous tissue shows healing with development of a fibrous barrier. Absence of inflammatory cells. (H&E stain, 10X)

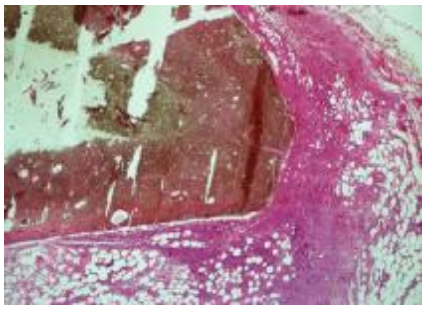


Figure no. 4. MTA at 21 days. Tendency to tissue healing and absence of the inflammatory infiltrate. The implant was rarefied due to macrophages activity and a thin fibrous capsule is developed. (PAS stain, 20X)

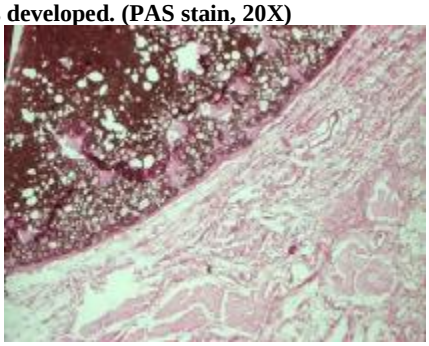
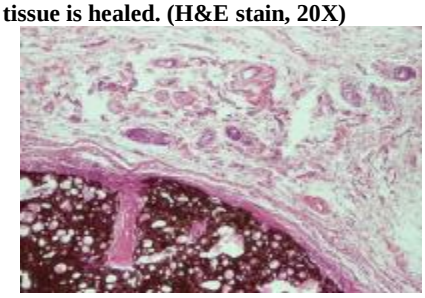


Figure no. 5. Life cement at 21 days. The thick fibrous barrier with a septum surrounds the implant and the adjacent tissue is healed. (H&E stain, 20X)



DISCUSSIONS

Direct pulp capping was the method of choice in young patients with traumatic pulp exposure who come for treatment during the first 24 hours. In these conditions, the correct control of hemorrhage, isolation and coverage of the defect with a biocompatible material under a tight seal offers best condition for healing by new dentin bridge formation. Therefore, the ideal pulp capping material has to adhere strongly to dental hard tissues, prevent microleakage, be insoluble in oral fluids, have a bactericidal effect and, finally yet importantly, be biocompatible.(7-11)

The most used material for pulp capping procedures is calcium hydroxide, which has a strong antibacterial effect and stimulates neodentinogenesis. In direct contact with vital tissue, it will determine a limited zone of necrosis, due to a pH of 11-12 in freshly mixed state. The healing process will start under this scar only if there is no microbial infiltration from the oral cavity.(12-14) Recently, many clinical studies presented very good results after pulp capping of immature teeth with MTA, which is now considered superior to $Ca(OH)_2$ regarding biocompatibility and hard tissue formation.(15)

MTA has an initial pH of 10.2, which rises to 12.5 after 3 hours; applied on dental pulp it induces the release of inflammatory cytokines and development of a hard barrier that resembles hydroxyapatite.(16-17) Therefore, one can say that MTA induces cellular and functional mechanisms at the surface of pulp tissue, offering an active biologic substrate with strong neodentinogenetic effect.(18-20)

Our investigation was based on an experimental method widely used in *in vivo* studies and is currently considered the most accurate approach in evaluating the degree of inflammation induced by different dental materials used in modern dentistry.

Regarding the effect of surgical procedure upon experimental animals, we noticed no difficulty in the placement of subcutaneous samples, which was easily done by a skin incision, and the general health and behavior was not affected by the presence of these implants. After 7 days, the histological examination revealed the development of inflammatory reactions at the control group and the study groups with Life cement, MTA and Calxyd paste, ranked as intense (2), and severe (3), respectively.

At 14 days postoperatively, the degree of inflammation showed clear signs of reduction; we noticed tissue healing and therefore all samples from study groups were ranked 1, corresponding to moderate inflammation. The common feature of tissue reactions were a clear demarcation of an inflammatory zone, surrounded by a fibrous capsule with many fibroblasts, which is considered a normal response reaction to subcutaneous implants. There was also a localized inflammatory infiltrate.

After 21 days, the clinical examination revealed a scar tissue, which exerted no pain on palpation. The surgical exposure of the implant showed that it became attached to the skin by a fibro-conjunctive transparent capsule, with a mild congestive reaction.

CONCLUSIONS

Subcutaneous implants of dental pulp capping materials such as MTA, Life cement, Calxyd by a surgical procedure do not determine alterations of general state of health of the experimental animals.

The inflammatory tissue reactions observed after implantation of MTA and Life cement are comparable, demonstrating that there are no significant differences in biocompatibility of these materials.

Anyway, this is not enough to rule out calcium hydroxide from every day dental practice, due to its excellent results.

Further clinical studies are necessary in order to evaluate the potential of MTA to induce dentin bridge formation on exposed dental pulp and its long-term clinical outcome.

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