SUBCUTANEOUS TISSUE REACTIONS TO MTA, PORTLAND CEMENT AND AN EXPERIMENTAL CEMENT

Reações tissulares ao MTA, cimento Portland e a um cimento experimental

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Abstract

Objectives: To evaluate the response of rat subcutaneous tissue to MTA, Portland cement and White Portland cement plus epoxy resin. Due to similarities in composition of MTA and Portland cement, white Portland cement was mixed with epoxy resin in order to obtain a more consistent material which would be easier to work with during clinical procedures.Methodology: MTA Angelus and Type II Portland Cement were mixed with distilled water in a 3:1 powder-liquid proportion. White Portland cement was mixed with epoxy-resin in the same proportion. The materials were placed into polyethylene tubes and implanted on the back of Wistar rats. Implants of empty tubes were used as control. Animals were kiled at 7, 30, and 60 days after implantation, and samples of the skin containing the tubes were removed and histologically processed. Results: At 7 days, a granulomatous tissue was observed nearby the extremity of the tube, characterized by lymphocytes, multinucleated giant cells, and abundant macrophages around a necrotic area. At 30 days, the tissue around the tube presented initial signs of repair characterized by fibrocytes and collagen fibers. A chronic inflammatory infiltrate characterized by many macrophages and some lymphocytes was also detected. At 60 days, an advanced process of repair was observed. No statistical differences were observed among the groups at 7 and 60 days ($p>0,05$); However some differences were seen at 30 days. More necrotic areas with granulomatous tissue and fibrosis were observed in Portland cement group when compared to the other cements ($p<0,05$). Lymphocytes were seen in much larger numbers in White Portland cement + epoxi resin group ($p<0,05$) (Kruskal-Wallis). Conclusions: The materials were not irritant to the tissues in the area, noticeably in the last periods, and did not interfere with the natural process of healing. Further studies are necessary to indicate Portland cement for clinical use, whereas MTA is already a routinely used product in endodontics.

Keywords: Portland cement; Mineral trioxide aggregate; Oral pathology.

Resumo

Objetivos: Avaliar a reação dos tecidos subcutâneos do rato ao MTA, cimento Portland e cimento Portland branco acrescido de resina epóxica. Devido às similaridades na composição entre o MTA e cimento Portland, o cimento Portland branco foi misturado com resina epóxica para obter um material mais consistente, o qual poderia ser de manipulação mais fácil durante os procedimentos clínicos. Metodologia: O MTA e cimento Portland foram misturados com água destilada na proporção de 3:1 (pó/ líquido). O cimento Portland branco foi misturado com resina epóxica na

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mesma proporção. O material foi colocado em tubos de polietileno e implantados no dorso de ratos Wistar. Tubos vazios foram utilizados como controle. Os animais foram mortos aos 7, 30 e 60 dias e amostras da pele contendo os tubos foram removidos e processados para exame histológico. Resultados: aos 7 dias, um tecido granulomatoso foi observado próximo à extremidade do tubo, caracterizado por linfócitos, células gigantes multinucleadas e abundantes macrófagos em torno de uma área necrótica. Aos 30 dias, o tecido em torno do tubo apresentava sinais iniciais de reparo, caracterizado por fibrócitos e fibras colágenas. Detectou-se igualmente um infiltrado inflamatório, caracterizado por muitos macrófagos e alguns linfócitos. Aos 60 dias, um processo avançado de reparo foi observado. Não houve diferenças estatísticas entre os grupos de 7 e 60 dias ($p>0,05$). Entretanto, algumas diferenças foram observadas aos 30 dias. Foram observadas mais áreas com tecido granulomatoso e fibróticas no grupo cimento Portland quando comparado com os outros cimentos ($p<0,05$). Linfócitos foram observados em número maior no grupo do cimento Portland acrescido de resina epóxica (p,0,05) (Kruskal-Wallis). Conclusões: Os materiais não foram irritantes na área estudada, principalmente nos maiores tempos de permanência e não interferiram com o processo natural de cicatrização. Estudos mais específicos devem ser conduzidos para indicar o cimento Portland para uso clínico, sendo que o MTA já é de uso rotineiro em Endodontia.
Palavras-chave: Cimento Portland; Agregado trióxido mineral; Patologia bucal.

Introduction

A mineral trioxide aggregate (MTA) was studied in a series of investigations, in vivo and in vitro. They reported good sealing ability (1,2) and tissue healing (3,4,5). Formation of new cementum over the material was reported in repair of experimentally perforated furcations (6), after root end filling (1,7,8) and root canal filling of dogs' teeth (9). Bridge-like dentin was observed in cases of pulp capping (5,10,11) and pulpotomy (3, 12) in monkey and dog teeth.

Wucherpfenning and Green (13) reported that both MTA and Portland cement type II seem almost identical macroscopically, microscopically and by X-ray diffraction analysis. They reported that both substances support matrix formation in a similar fashion in cultures of osteoblastlike cells, and also in apposition of reparative dentin when used as direct pulp capping material in rat teeth. Other studies (14,15) affirm that Portland cements contain the same chemical elements as MTA. Some of these ingredients are calcium phosphate, calcium oxide, and silica. However, MTA, but not Portland cement, also contains bismuth oxide, which increases its radiopacity.

Portland cement type II is a construction cement and yet presents great similarity to MTA, which might be interesting in a way it may offer significant economic incentives if applicable in biological systems. Previous studies have demonstrated good results when Portland cement was used as a pulp capping material (12) and in cell culture tests (16).

The idea of mixing Portland cement with an epoxy resin was to contribute to a better manipulation of the material, making it easier to use in any type of cavity. Considering its use in esthetic areas, as with white MTA, the purpose of this study was to evaluate the a mixture of white Portland cement and epoxy resin implanted in rat subcutaneous tissues, considering especifically if the addition of epoxy resin would contribute to an irritant behavior of the cement.

Material and methods

This study was carried out following the guidelines of the Ethics Committee for Teaching and Research in Animals of the Bauru Dental School University of São Paulo, Bauru, SP, Brazil. Thirty-six male Rattus norvegicus, weighing about 250g each, from the Central Animal Laboratory of Bauru Dental School, were randomly divided into 3 groups, according to the sealer and experimental period.

For each group, four rats were used for each of the three different experimental periods of 7, 30 and 60 days. Group 1 received implants containing MTA Angelus (Angelus, Londrina, PR, Brazil), Group 2 containing

Portland cement Type II (Votorantin, SP, Brazil) and Group 3 containing white Portland cement (Irajazinho, SP, Brazil), mixed with epoxy-resin. MTA Angelus and Type II Portland cement were mixed with distilled water in a 3:1 ratio. White Portland cement was mixed with epoxy resin also in a 3:1 ratio. The materials were mixed at the moment of implantation.

Preparation of animals and procedures for implantation

The same surgical sequence was used for all animals. The animals were anesthetized with an intramuscular injection of a mixture of ketamine/xylazine (Agribrands do Brasil, Paulinia, SP, Brazil) in a 1:1 ratio (v/v), 0.5 mL/ kg of weight. After asepsis of the area, two straight incisions of approximately 1.5 cm were made with a #10 blade (Becton-Dickson, Sao Paulo, SP, Brazil) exposing the subcutaneous connective tissue. Two polyethylene tubes (1 mm in diameter and 1 cm in length) previously desinfected in 5% sodium hypochlorite for 5 minutes and washed in deionized water for 15 minutes under sonification, were implanted on the back of the animal, one on each side. One tube was filled with one of the cements to be tested and the other was kept empty as control. The wounds were sutured. All animals received a normal diet and water ad libitum throughout the entire study.

The animals were anesthetized and a sample of the tissue containing the implants was removed at 7, 30 and 60 days after implantation. Thereafter, the animals were killed by cervical displacement, according to the guidelines of the Brazilian College of Animal Experimentation (COBEA). The specimens were fixed in buffered 10% formalin for 24 hours. After histotechnical processing, 5µm thick sections were taken and stained with hematoxylin-eosin.

Microscopic analysis

For the evaluation of the chronic inflammatory reaction of subcutaneous connective tissue, necrotic or granulomatous tissue, presence of macrophages, multinucleated giant cells, lymphocytes, granulation or fibrous tissue, were observed and classified as absent, discrete, moderate and intense by two observers previously calibrated, in relation to numerical scores of 0, 1, 2 and 3, respectively.

Results

The results of the microscopic analysis were submitted to non-parametric Kruskal-Wallis test and are presented in Table 1. Mean values for each parameter evaluated for each of the materials in the different time periods tested. Kruskal-Wallis was used for statistical analysis. Dunn's multiple comparison test was used when $p<0,05$.

Table 1 - Itens observed in optical microscopic analysis

Period (days)		Group 1 Mean	Group 2 Mean	Group 3 Mean	p
7	Necrosis	2,0	2,0	2,0	p>0,05
	Granulomatous tissue	2,0	2,25	2,75	p>0,05
	Macrophages	3,0	3,0	3,0	p>0,05
	Giant cells	0,5	0,5	0,5	p>0,05
	Limphocytes	1,0	1,0	1,25	p>0,05
	Process of repair	0	0	0	p>0,05
	Fibrosis	0	0	0	p>0,05
30	Necrosis	1,0 [a]	0 [b]	1,0 [a]	P<0,05
	Granulomatous tissue	1,0 [a]	0 [b]	1,0 [a]	P<0,05
	Macrophages	2,0	2,0	2,0	p>0,05
	Giant cells	0,25	0	0	p>0,05
	Limphocytes	0 [b]	0 [b]	1,0 [a]	p<0,05
	Process of repair	2,0 [a]	3,0 [b]	2,0 [a]	p<0,05

	Fibrosis	1,0 [a]	2,0 [b]	1,0 [a]	p<0,05
60	Necrosis	0,5	0,5	0,5	p>0,05
	Granulomatous tissue	0,25	0	0	p>0,05
	Macrophages	1,25	1,5	1,0	p>0,05
	Giant cells	0	0,5	0	p>0,05
	Limphocytes	0,25	0	0	p>0,05
	Process of repair	2,75	3,0	3,0	p>0,05
	Fibrosis	2,0	2,0	2,0	p>0,05

* sealers with the same reference letter "a" or "b" did not present any statistical difference.

No statistical differences were observed among the groups at 7 days (p>0,05); however, some differences were seen at 30 days. More necrotic areas with granulomatous tissue and fibrosis were observed in group 2 when compared to the other cements (p<0,05). Lymphocytes were seen in much larger numbers in group 3 (p<0,05). At 60 days all groups showed the same tissue response not presenting significant statistical differences in any of the parameters observed. Areas of fibrotic tissue nearby the extremity of the tubes, suggesting an ongoing repair process was observed in all groups.

Group 3 demonstrated similar and satisfatory tissue reactions (Figures 1, 2, and 3).

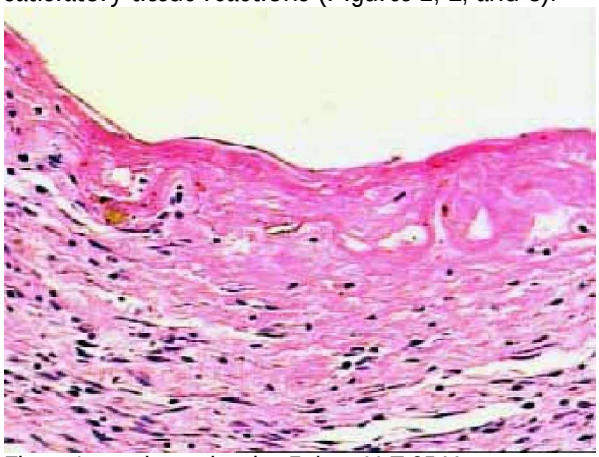

Figure 1 experimental sealer, 7 days, H.E 25 X.

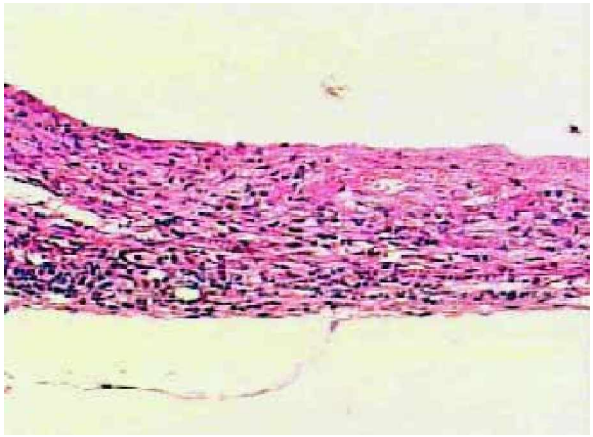

Figure 2 experimental sealer, 30 days, H.E 25 X.

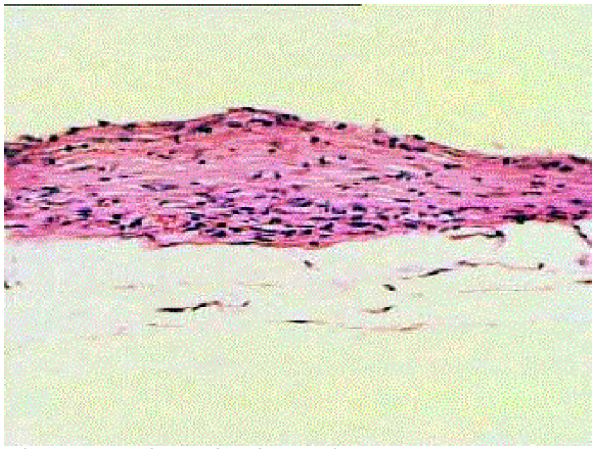

Figure 3 experimental sealer, 60 days, H.E 25 X.

The control group demonstrated less necrotic areas, fewer inflammatory cells and larger areas of fibrotic tissue formation throughout the experimental periods.

Discussion

MTA has many indications in endodontics. According to previous studies (3, 12, 16), Portland cement appears to be an alternative to MTA since the biological effects are identical. It is a quite inexpensive and an easily available material. In a recent study, attempting to enhance the setting time of Portland cement, Abdullah et al (17) observed that one type of accelerated Portland cement (APC) is non-toxic and may have the potential to promote bone healing. The authors also stated that further testing of APC is needed to produce a viable dental restorative material and possibly a material for orthopedic purposes.

We recently reported good results obtained using Portland and White Portland cement in pulpotomies in dog's teeth (12), and have been trying to improve the manipulation of the material, mainly due to difficulties in filling small cavities.

Many types of Portland cement are available on the market. Nowadays, there are several substances that can be added to Portland cement, providing greater resistance, impermeability, and a faster setting time. In this study we used Portland cement type II, for being easily obtained.

The idea of mixing Portland cement with an epoxy resin was to contribute to a better manipulation of the material, making it easier to use in any type of cavity. Nevertheless this procedure could contribute to a less biocompatible behavior of the cement. Surprisingly this was not seen in our results, probably because of the minimal amount of epoxy resin and also because of the initial setting time, about 5 minutes. We chose to use White Portland mixed to epoxy resin because it could be used in esthetic areas.

Specifications determined by the American Dental Association since 1972 consider implant methodologies as valuable methods in preliminary studies of tissue compatibility. Other studies evaluated the rat subcutaneous tissue reactions to verify the biocompatibility of materials, through the insertion of polyethylene tubes (18,19) or dentin tubes (4) implantation or injection (20). A great disadvantage of such method is the movement of the tube within the tissue (21).

The tissue responses, especially in the longer experimental periods, demonstrated similar behavior of the materials tested, favoring the formation of a fibrotic tissue nearby the end of the tubes. It is an attempt by the tissue to prevent the spread of the disease or agression. The presence of a fibrotic tissue at a previously affected area suggests a process of repair, problably by the remission of any aggressive action of the materials in contact with the tissue, thus allowing fibroblasts to initiate a process of synthesis of collagen.

Further studies with longer observation periods should be realized, such as the hard tissue implant technique to verify the mineralization of the tissue, which would be ideal in retrocavities. Even though considered preliminary only, the findings of this study can be considered satisfactory especially when it comes to a material with many potential applications and low cost.

Nevertheless, other clinical studies and considerations of the limitations and the potential unknown risks involved in the use of building materials as medical devices are necessary to define their safe use.

References

1. Torabinejad M, Rastegar F, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. J Endod, 1995; 21:109-112.

2. Aqrabawi J. Sealing ability of amalgam, super EBA cement, and MTA when used as retrograde filling materials. Brit Dent J 2002; 188: 266-268.

3. Holland R, Souza V, Murata SS, Nery MJ, Bernabé PFE, Otoboni Filho JA, et al. Healing process of dog dental pulp after pulpotomy and pulp covering with mineral trioxide aggregate or Portland cement. Braz Dent J, 2001;12: 109-113.

4. Holland R, Souza V, Nery MJ, Otoboni Filho JA, Bernabe PFE, Dezan Junior E. Reaction of rat connective tissue to implanted dentin tubes filed with mineral trioxide aggregate or calcium hydroxide. J Endod 1999; 25:161-166.

5. Pitt Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasan SP. Using mineral trioxide aggregate as a pulp-capping material. J Am Dent Assoc 1996;127:1491-1496.

6. Pitt Ford TR, Torabinejad M, McKendry DJ, Hong CU, Kariyawasam SP. Use of mineral trioxide aggregate for repair of furcal perforations. Oral Surg Oral Med Oral Patho 1995; 79: 756-762.

7. Torabinejad M, Hong CU, Lee SF, Monsef M, Pitt Ford TR. Investigation of mineral trioxide aggregate for root-end filling in dogs. J Endod, 1995; 21:603-8.

8. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod, 1993; 19:591-595.

9. Holland R, Souza V, Nery MJ, Otoboni Filho JA, Bernabé PFE, Dezan Jr. E. Reaction of dog´s teeth to root canal filling

with mineral trioxide aggregate or a glass ionomer sealer. J Endod, 1999; 25:591-595.

10. Faraco Jr. IM, Holland R. Response of the pulp of dogs to capping with mineral trioxide aggregate or a calcium hydroxide cement. Dental Traumatol 2001;17:163-166.

11. Tziafas D, Pantelidou O, Alvanou A, Belibasakis G, Papadimitriou S. The dentinogenic effect of mineral trioxide aggregate (MTA) in short-term capping experiments. Int Endodon J 2002; 35:

12. Menezes R, Bramante CM, Letra A, Carvalho VG, Garcia RB. Histologic evaluation of pulpotomies in dog using two types of mineral trioxide aggregate and regular and white Portland cements as wound dressings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2004; 98:376-379.

13. Wucherpfennig AL, Green DB. Mineral trioxide vs Portland cement: two biocompatible filling materials. J Endod 1999; 25:308 (Abstract).

14. Estrela C, Bahmann LL, Estrela CRA, Silva RS, Pécora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. Braz Dent J 2000; 11:19-27.

15. Funteas UR, Wallace JA, Fochtman EW. A comparative analysis of Mineral Trioxide Aggregate and Portland cement. Aust Endod J 2003; 29: 43-44.

16. Saidon J, He J, Zhu Q, Safavi K, Spangberg LSW. Cell and tissue reactions to mineral trioxide aggregate and Portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95:483-489.

17. Abdullah D, Ford TR, Papaioannou S, Nicholson J, McDonald F. An evaluation of accelerated Portland cement as a restorative material. Biomaterials 2002; 23: 4001-4010.

18. Torneck CD. Reaction of rat connective tissue to polyethylene tube implants. I. Oral Surg Oral Med Oral Pathol. 1966;21: 379-87.

19. Orstavik D, Mjor IA. Histopathology and x-ray microanalysis of the subcutaneous tissue response to endodontic sealers. J Endod. 1988;14;13-23.

20. Safavi KE, Pascon EA, Langeland K. Evaluation of tissue reaction to endodontic materials. J Endod. 1983;9;421-9.

21. Olsson BO, Sliwkiwski A, Langeland K. Subcutaneous implantation for the biological evaluation of endodontic materials. J Endod., 1981; 7:355-369.

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