Comparison of Skin Test Reactivity of Two Endodontic Biomaterials in Rabbits

Bahareh Tabarsi, 1Mohsen Pourghasem, 1Aliakbar Moghaddamnia, 1Marziyeh Shokravi, 1Maryam Ehsani, 1Maryam Ahmadyar and 1Saeed Asgary
1Cellular and Molecular Research Center, Dental School, Babol University of Medical Sciences, Babol, Iran
2Department of Anatomical Sciences, Babol University of Medical Sciences, Babol, Iran
3Department of Pharmacology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran
4General Dentist, Babol, Iran
5Dental Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
6Iranian Center for Endodontic Research, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract: In this in vivo study, the skin reactivity of rabbits to Mineral Trioxide Aggregate (MTA) and Calcium Enriched Mixture (CEM) was compared. Sixteen albino rabbits were used. The dorsal skin in three areas (2×2 cm) of the rabbits was shaved 24 h prior to the test. The areas were randomly covered with freshly mixed biomaterials [MTA (n = 16), CEM (n = 16) and normal saline (control; n = 16)], sterile gauze and bandages were placed for 4 h and the biomaterials were washed. The surface areas of the reactive skin sections were examined by naked eye and measured in 1, 24, 48 and 72 h using the Cavalieri technique. The animals were then sacrificed and histological sections were prepared for blind examination. Results of macroscopic examination revealed a significant difference (p = 0.003) between the average erythematosus surface areas induced by MTA (1.61±0.32) and CEM (0.5±0.16). Results of histological examination showed that the highest inflammation was observed in MTA, followed by CEM and control groups. Comparison of the difference in inflammatory cell count between each group revealed statistically significant differences in all cases (p<0.001). The results demonstrated that the biocompatibility of CEM cement is higher than MTA; CEM can be considered as a suitable endodontic biomaterial.

Key words: Biocompatibility, calcium enriched mixture, CEM cement, endodontics, mineral trioxide aggregate, skin reaction

INTRODUCTION

Dental caries remains one of the most common human diseases throughout the world (Motalgh et al., 2007; Malhi et al., 2006). The underlying dental pulp may undergo iatrogenic/pathological damage/changes caused by caries or even periodontal diseases and require various treatments such as vital pulp therapy, repair of perforations, apical surgery and apexification. Because the materials used for such procedures are in direct contact with living tissues, they should have adequate biocompatibility (Torabinejad and Chivian, 1999).

Mineral Trioxide Aggregate (MTA) contains Portland cement, calcium sulfate and bismuth oxide. MTA has excellent sealing ability, minimal cytotoxicity and high tissue biocompatibility (Parirokh and Torabinejad, 2010a, b). Another advantage of MTA as a hydrophilic cement is that complete moisture control and arrest of pulp hemorrhage is not necessary during treatment (Torabinejad et al., 1994). It also appears that MTA has the ability to induce hard tissue formation by cementoblasts (Torabinejad et al., 1997). MTA has a pH of 10.2 when freshly mixed which increases to 12.5 after 3 h (Torabinejad et al., 1995). However, MTA also has some disadvantages such as difficult handling characteristics, delayed setting time (4-6 h), high cost and the need for two visit treatment (Torabinejad and Parirokh, 2010).

Calcium Enriched Mixture (CEM) cement has been recently introduced (by the last author; Patent as Endodontic Filling Material, USPTO Number: 7, 942, 961) as a new endodontic biomaterial (Asgary and Kamrani, 2008). CEM contains various calcium compounds and has similar applications to MTA although, it is different in terms of its chemical

Corresponding Author: Mohsen Pourghasem, Department of Anatomical Sciences, Babol University of Medical Sciences, Babol, Iran

250
components (Asgary et al., 2008c; Asgary et al., 2009b). This material has acceptable physical properties; its setting time is less than 1 h, it is easy to handle and costs relatively less than MTA (Asgary et al., 2008c). Properties of CEM that are comparable to MTA include: sealing ability (Asgary et al., 2008a), hard tissue induction (Samiee et al., 2010; Tabarsi et al., 2010), dentinogenesis (Asgary et al., 2008b; Nosrat and Asgary, 2010), cementogenesis (Asgary et al., 2010) and low cytotoxicity (Moayyedi et al., 2010); whilst properties superior to MTA include: antibacterial activity (Asgary and Kamrani, 2008) and hydroxyapatite formation in saline solution (Asgary et al., 2009a).

The aim of the present in vivo study was to assess the biocompatibility of two endodontic biomaterials by conducting skin reactivity tests using an animal model.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Dental Research Centre of Shahid Beheshti Medical University, Tehran, Iran. Animals were housed in accordance with international recommendations. Sixteen healthy male New Zealand Albino rabbits with an average weight of \( \approx 2 \) kg were used. The animals were kept under climate controlled laboratory conditions of 12 h light/darkness cycles, 25-35°C temperature and 50-55% moisture.

Three areas measuring 2\( \times \)2 cm with 2 cm distance from each other were randomly selected on both sides of the animals’ spines; one area was set as control and two areas were experimental. The dorsal skin in these areas was shaved (Mouzer machine, Germany) and disinfected using 10% betadine solution (Darou Paksh, Tehran, Iran). After 24 h, the materials [Tooth-coloured ProRoot MTA (Dentsply Tulsa Dental, Tulsa, Ok, USA) and CEM (BioniqueDent, Tehran, Iran)] were mixed according to manufacturers’ instructions and placed randomly in a uniform layer onto the shaved areas by complying with international standards (ISO 10993-10); normal saline was used as control. The areas were then covered with moist gauze and bandages. After 4 h, the biomaterials were washed using saline irrigation. The erythematous skin surfaces of the experimental and control areas were then evaluated with naked eye by a blind examiner at intervals of 1, 24, 48 and 72 h. The quantitative analytical Cavalier technique was used to calculate the total surface areas which were representative of skin reactivity.

The animals were then sacrificed after the final observation and tissue samples were removed for histological examination. The tissue samples were fixed in 10% buffered formalin, subjected to routine histological processing and embedded in paraffin. Then \( \approx 5 \mu m \) thick serial longitudinal sections were obtained from the central portion of each sample. One blinded pathologist evaluated the specimens in a blind fashion using a light microscope (Olympus, Tokyo, Japan); images were captured using a Moticon digital camera (x400 Mag.). The prepared sections were then examined for the average number of inflammatory cells in the experimental and control areas in all of the animals.

Statistical analysis: Data analysis was carried out by repeated measure, ANOVA with sphericity assumption and statistical significance was set at 5% for all analyses. Analysis of results was done using SPSS (Version 13) statistical package.

RESULTS

Results of macroscopic eye examination of erythematous skin areas showed that irrespective of time, there was a significant difference between the average erythematous surface areas induced by MTA (1.61±0.32) compared to CEM (0.5±0.16) (p = 0.003; Table 1). Irrespective of the material used, there was a significant difference in total average erythematous surfaces of the three areas at different time intervals (p = 0.006). This statistical difference was observed in paired comparisons between 1, 24, 48 and 72 h observation periods (p = 0.014, 0.007 and 0.01, respectively). Further paired comparisons between other time intervals did not reveal significant differences. In addition, analysis of confounding effects of the materials used and time elapsed on the erythematous areas did not reveal a statistically significant difference (p = 0.467).

Estimated marginal means of erythematous surface areas induced by the experimental biomaterials over time are presented in Table 1. Results of histological examination revealed that the highest inflammatory cell count was observed in MTA (8.13±2.53), whereas in the case of CEM (6.44±1.25) the situation was very similar to the control areas (5.1±1.51) (Fig. 1).

Table 1: Estimated marginal means of erythematous surface areas induced by the experimental biomaterials (n = 16)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (h)</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEM</td>
<td>1</td>
<td>0.08±0.0159</td>
</tr>
<tr>
<td>CEM</td>
<td>24</td>
<td>0.57±0.2534</td>
</tr>
<tr>
<td>CEM</td>
<td>48</td>
<td>0.56±0.2507</td>
</tr>
<tr>
<td>CEM</td>
<td>72</td>
<td>0.74±0.0576</td>
</tr>
<tr>
<td>MTA</td>
<td>1</td>
<td>1.15±0.142</td>
</tr>
<tr>
<td>MTA</td>
<td>24</td>
<td>1.89±0.1407</td>
</tr>
<tr>
<td>MTA</td>
<td>48</td>
<td>1.77±0.1355</td>
</tr>
<tr>
<td>MTA</td>
<td>72</td>
<td>1.69±0.142</td>
</tr>
</tbody>
</table>

251
DISCUSSION

Biocompatibility is one of the most important factors in choosing medical devices (Jafari et al., 2007; Rezaei-Taviram et al., 2008; Ouedraogo et al., 2008a-b; Abbassalipourkabir et al., 2011; Sahu et al., 2011; Mobarak, 2011; Mishebuko et al., 2011), particularly an endodontic biomaterial in order to prevent any adverse reactions which may lead to treatment failure (Zmener, 2004). The skin reactivity test is a simple method which can be used as a first step assessment of tissue biocompatibility of a material. Considering that endodontic materials lie adjacent to the pulp and the tissues surrounding the root, a more severe reaction is expected in those tissues compared to the skin. In this study, the amount of skin reactivity (measured in terms of average erythematous surface areas and histological assessments) to MTA and CEM revealed that the tissue biocompatibility of CEM was higher than MTA.

In the present study, assessment of skin reactivity was carried out by measurement of the inflamed erythematous areas using Cavalier analysis in order to achieve more reliable data. In order to simplify reporting of the results of histopathological assessment, comparison of the amount of inflammation and average number of inflammatory cells induced by the materials was carried out.

The results of histological examination in the present study were similar to previous studies which used subcutaneous or direct placement of MTA on vital pulp tissue of the tooth (Moreton et al., 2000) assessed the biocompatibility of MTA by subcutaneous and intraosseous placement of the material during 15, 30 and 60 day time intervals. MTA showed an initial severe inflammatory reaction with coagulative necrosis and the inflammation reduced with time (Moreton et al., 2000). Similar results were observed in a number of other studies (Yaliturk et al., 2004; Sumer et al., 2006). Although, the above studies included a longer follow-up period compared to the present study, a similar overall trend was also observed in our study.

In the present study, a less severe tissue reaction to MTA was observed which is indicative of the relatively high biocompatibility of MTA (Eghbal et al., 2009; Mozayeni et al., 2010) although, CEM was found to have higher biocompatibility compared to MTA.

One theory for induction of inflammation by MTA may be due to the fact that MTA has an initial pH of 10.2 which reaches 12.5 after 3 h (Torabinejad et al., 1995). Therefore, the high surface pH of freshly mixed MTA may lead to denaturation of tissue proteins in the surrounding cells. Although, the initial pH of CEM is similar to MTA,

Fig. 1: Average numbers of inflammatory cell count in experimental and control areas

Fig. 2: Light microscope photographs of histological samples showing lower inflammatory cell count in CEM compared to MTA samples

Analysis of the difference in inflammatory cell count between each group revealed a statistically significant difference in the average numbers of inflammatory cells between all three groups {CEM-MTA (p = 0.028), CEM-Control (p = 0.008) and MTA-Control (p = 0.001)} (Fig. 2).
there have not been any recorded significant changes in pH of CEM over time, although further research is required in this area (Ghazvini et al., 2009).

Researchers have demonstrated that when MTA and CEM are in contact with PBS (containing phosphate ions), they produced hydroxyapatite crystals. This process was not observed in the MTA samples immersed in normal saline; however, CEM immersed in saline solution showed presence of hydroxyapatite crystals (Asgary et al., 2008c). Therefore, it can be concluded that unlike MTA, CEM can be considered as a source of calcium and phosphate ions which react to form hydroxyapatite crystals; this will reduce the number of free ions at the tissue interface which in turn leads to a decrease in pH.

CONCLUSION

It appears that the tissue reactivity to MTA is higher compared to CEM; therefore, it can be concluded that in the short term, the biocompatibility of CEM is superior to MTA.

ACKNOWLEDGMENTS

This study was supported by a grant from Iranian Center for Endodontic Research, Shahid Beheshti University of Medical Sciences, Tehran and Molecular Research Center, Babol University of Medical Sciences, Babol, Iran.

REFERENCES


