The Influence of Gross Caries Removal and Temporary Filling of Dental Caries with a Zinc Oxide Eugenol Cement on the Level of Mutans Streptococci in Saliva

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The influence of gross caries removal and temporary filling of dental caries with a zinc oxide eugenol cement on the salivary level of mutans streptococci (MS) was studied in 14 children, 6 to 8 years of age. Forty-eight hours after filling all cavities, there was a significant decrease in the level of these cariogenic microorganisms. However, after 30 days, the salivary level of mutans streptococci was similar to that detected initially.

Key Words: mutans streptococci, zinc oxide eugenol cement, temporary fillings.

Introduction

The restoration of carious lesions with zinc oxide eugenol cement is a recommended technique (Abramowicz and Araújo, 1988) and is routinely adopted by pediatric dentists and general dentists.

According to Mondelli et al. (1976), the indirect pulp capping objectives are: block the aggressions which affect the pulp through the lesion, interrupt the metabolic circuit between the oral cavity and the decayed tissue, inactivate the dental caries progression by the bactericidal/bacteriostatic action of the capping material, and remineralize the remaining affected dentin. In pediatric dentistry, the temporary filling of carious lesions allows the professional a longer time for psychological preparation of the patient for dental treatment, as well as for an individualized preventive program.

Elliot (1964) observed a salivary lactobacilli level reduction of 76.8% in children, one week after complete oral rehabilitation with amalgam restorations.

Abramowisk and Araújo (1988) reported that zinc oxide eugenol fillings reduced cariogenic activity for 2 months, evaluated by Snyder test and lactobacilli count. However, they observed that after this time, the number of lactobacilli returned to initial levels.
Mutans streptococci (MS) are recognized as the most cariogenic microorganisms due to their metabolic activities. These bacteria play an important role in dental decay etiopathogenesis (Loesche, 1986). MS salivary levels have been used in the determination of the risk of caries in patients (Krasse, 1986). Various aspects of ecology and colonization of S. mutans have been studied recently (Loesche, 1986) and it is known that, even in caries-free individuals, MS colonize the occlusal and approximal surfaces, which have a greater risk of developing caries (Loesche, 1986). The presence of retention sites in the oral cavity, such as orthodontic appliances (Scheie, 1984), removable partial dentures (Mihallow and Tinnonoff, 1988) and open carious lesions (Loesche and Syed, 1973), can act as reservoirs for these bacteria which are constantly released into saliva.

Keene et al. (1976) examined the prevalence of S. mutans in dental plaque from carious and noncarious sites before and after routine dental treatment in 5 US Navy recruits. After all carious lesions had been restored with silver amalgam, the level of S. mutans was significantly reduced, but many of the sites still had detectable levels of this organism.

Loesche et al. (1977), studying the effect of kanamycin gel treatment on the S. mutans level in patients with rampant caries, submitted 45 individuals (4-17 years of age) to restorative treatment using silver amalgam and stainless steel crowns. The salivary level of S. mutans was significantly reduced in the placebo group (gel without kanamycin) two weeks after restorative treatment. However, one week later, there was a considerable increase in the level of this bacteria.

Due to the participation of MS in the etiopathogenesis of dental caries, of the clinical importance of gross caries removal and temporary filling of carious lesions and of the lack of studies correlating these microorganisms with the filling of open cavities, the objective of this study is to verify the influence of gross caries removal and temporary filling of the decayed tissue with a zinc oxide eugenol cement on the level of MS in the saliva of children.

Material and Methods

Fourteen children, aged 6-8 years, who sought treatment at the Pediatric Dental Clinic of the School of Dentistry of Ribeirão Preto, University of São Paulo, Brazil, were chosen to participate in this study. They presented 4-8 posterior teeth with carious lesions which allowed the retention of the filling material. These patients did not present necrotic teeth and/or furca lesion, suggested by the radiographic examination.

The carious lesions were cleaned using spoon excavators to remove the maximum amount of decayed tissue as possible, especially of the surrounding walls. After relative isolation of the field with cotton rolls and drying, pulp protection with a calcium hydroxide paste [Ca(OH)₂ - Merck, and distilled water] was performed, and then the temporary filling with a zinc oxide eugenol cement [zinc oxide p.a. Reagen, and eugenol - Dierberger, + 1% zinc acetate] was done. In cases of deep caries, with risk of pulp exposure, regional anesthesia and rubber dam isolation were done; and whenever pulp exposure had already occurred, pulpotomy was performed followed by the application of 1/5 diluted formocresol
for 5 minutes. The pulp chamber and the whole tooth cavity were then filled with the zinc oxide cement.

Neither the patients nor their parents received oral hygiene or diet instructions during the study.

Unstimulated saliva samples were collected for 5 minutes in sterilized test tubes with 4-5 glass beads, before (1st sampling), 48 hours (2nd sampling), 1 week (3rd sampling) and 1 month (4th sampling) after the gross caries removal and the filling of all cavities, which were performed in a single visit.

After each sampling, always done at the same time of the day for each patient, the tubes were immediately refrigerated and sent for microbiological processing.

In the laboratory, the saliva samples were homogenized in a Vortex mixer for 30 s, diluted in a phosphate buffered solution and spread with the aid of "L" shaped glass rods in petri plates containing an MS selective medium - SB-20 agar (Azevedo, 1988). After 72 h incubation at 35°C, in an anaerobic atmosphere (Gas Park - BBL), the determination of the number of colony forming units (CFU) was performed using a stereoscopic microscope.

Results

Mutans streptococci were detected in the saliva of all of the children at the 1st sampling (before filling), with levels ranging between $1.8 \times 10^5$ and $7.55 \times 10^6$ CFU/ml saliva (mean $2.76 \times 10^6$). At the 2nd sampling (48 hours after filling) it was not possible to detect MS in 6 patients, with a mean of $7.8 \times 10^4$ CFU/ml saliva. At the 3rd (1 week after) and the 4th samplings (1 month after) a mean value of $5.42 \times 10^5$ and $6.11 \times 10^6$ CFU/ml saliva were found (Table 1).

These data were analyzed by the Wilcoxon test, a non-parametric method for the comparison of samples from the same individual.

Figure 1 shows that 48 hours after filling of the carious lesions (2nd sampling) there was a reduction in the MS levels which was statistically significant ($P < 0.01$). An increase in the number of MS in the saliva of various children was observed after 7 days; however, not statistically significant in relationship with that found at the 2nd sampling. One month after filling (4th sampling), the patients presented MS levels statistically similar to those detected at the 1st sampling and higher than those of the 3rd sampling ($P < 0.05$), suggesting the tendency to reestablishment of the saliva level of these bacteria as a function of time.

Discussion

Despite the systematic removal of the decayed tissue, followed by temporary filling or restoration of the cavities being considered an effective method of disease control (Massler, 1981), there is little clinical or experimental evidence supporting this concept, since strictly restorative treatment offered by dentists has contributed little to a decline of dental caries prevalence (Pinto, 1990) or of the cariogenic potential of the patients (Assis et al., 1990).
Table 1 - Mutans streptococci levels (CFU/ml) detected in the saliva of children.
ND, Not detected. *, Patient did not appear for sampling.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sampling</th>
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<tbody>
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<td>1</td>
<td>7.97 x 10^5</td>
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<td>2</td>
<td>3.90 x 10^5</td>
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<td>3</td>
<td>2.49 x 10^6</td>
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<td>7</td>
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<td>3.60 x 10^6</td>
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<td>14</td>
<td>3.45 x 10^5</td>
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<td>Mean</td>
<td>2.76 x 10^6</td>
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Figure 1 - Graphical presentation of mean levels of MS in the saliva of 14 children in function of time.
On the other hand, Kesel et al. (1958) and Elliot (1964) have reported changes in oral microbiota after routine clinical dental procedures, especially in the levels of lactobacilli, yeasts, staphylococci and streptococci. Reduction in the number of several of these potentially cariogenic microorganisms could lead us to infer that restorative treatment is capable of reducing cariogenic potential.

Carious lesions are not the primary sites of MS colonization; however, they may retain these microorganisms (Loesche and Syed, 1973), contributing to the constant re-infection of the patient's mouth and consequently maintain the microbiological risk of development of new lesions (Krasse, 1986). The elimination of these reservoirs, by means of restoration, despite partially eliminating these bacteria from the dental plaque adjacent to the lesion, does not result in a complete eradication of MS (Keene et al., 1976).

Massler (1981) recommends the preparation of the patient's oral cavity, before restorative treatment, by means of provisional filling of the carious lesions with temporary cement in order to protect the subjacent dental pulp and also to remove niches of microbial retention, consequently decreasing the risk of new carious lesions. However, this filling, in spite of being suggested and performed for several years, is not scientifically based on the Specific Plaque Hypothesis (Loesche, 1986).

The results of this study show that filling carious cavities with zinc oxide eugenol cement affects the salivary level of MS, since 48 h after these temporary restorative procedures were done, there was a reduction in the number of these microorganisms. However, after 1 week the level of MS increased slightly and after 30 days there was no statistically significant difference compared to the 1st sampling (before filling).

Keene et al. (1976) showed that after dental caries are restored there is a reduction in the number of sites of S. mutans and also a decrease in the proportion of S. mutans to total streptococci. Removal of carious tissue followed by the restoration of cavities, however, did not result in complete elimination of S. mutans, demonstrating that restoration is an effective method, yet temporary, in the control of these cariogenic microorganisms.

Loesche et al. (1977) detected a decrease in the salivary level of S. mutans after restorative treatment. However, after 1 week, a statistically significant increase was noted.

The initial decrease in the MS level immediately after filling followed by an increase can perhaps be explained by the antimicrobial action of eugenol and/or zinc oxide (Tobias et al., 1985).

Svanberg et al. (1990) and Berg et al. (1990) have shown that the microbial composition of plaque, and consequently of saliva (Togelius, 1984), may be affected not only by filling of the carious cavities, but also by the action of the different dental materials used. According to Berg et al. (1990), the antimicrobial action lasts while the bactericidal/bacteriostatic substance of the material used is active (i.e. present and in minimal inhibitory concentrations).

The relative influence of the filling material on the level of these microorganisms is being studied by the substitution of this material by other similar ones.

Other hypotheses can be raised in an attempt to explain the increase in the level of MS, in a relatively short interval, after the filling of carious lesions. The high level of these
microorganisms detected on the 1st sampling (> 10^5 CFU/ml saliva) in all of the children is, according to Duchin and Van Houte (1978), essential for new colonization in favorable areas and, thus, ought to favor re-colonization. Associated with this, the surface irregularity of the temporary restorations may act as micro-sites of retention, creating ecologically favorable niches for a fall in pH and growth of MS, as well as other aciduric microorganisms such as lactobacilli.

The temporary reduction in the salivary level of MS observed in this investigation is similar to that described by Loesche et al. (1977) and Keene et al. (1976) illustrating the difficulty in controlling the risk of new caries with only restorative treatment. This suggests the necessity of further investigations seeking to associate preventive measures capable of increasing the resistance of enamel to demineralization and of reducing the cariogenic oral microbiota with gross caries removal and temporary filling of cavities.

Conclusions

The gross caries removal and temporary filling of carious lesions with a zinc oxide eugenol cement initially reduced the salivary level of MS; however, 30 days after treatment, the number of these microorganisms in saliva returned to the level detected before treatment.

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