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Original research

ASSESSMENT OF THE EFFECTIVENESS OF OZONE THERAPHY AND AN ANTIBACTERIAL BONDING AGENT ON THE CAVITY DISINFECTION OF DECIDUOUS TEETH: AN IN VIVO STUDY

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ABSTRACT

Objectives: The aim of this study is to evaluate antibacterial effectiveness of 30 second ozone therapy applied via OzonyTronX, Clearfil Protect Bond (a MDPB containing bonding agent) and Dycal ($Ca(OH)_2$ containing cavity lining material) on *Streptococcus mutans* in deciduos teeth.

Materials and Methods: 40 primary molars were obtained from ten patients whose ages ranged between 5 and 11. Dentin samples which were collected before the treatment and after a period of four weeks following the implementation of materials were microbiologically evaluated and material's antibacterial effectiveness were compared.

Results: Differences between the antibacterial effectiveness of the materials were found to be statistically significant according to the results of covariance analysis with randomized block design ($p<0.05$). Antibacterial effectiveness of the groups on *S. mutants* is Group 2 (ozone therapy)> Group 3(CPB)> Group 1(Dycal).

Conclusions: Although CPB, which is an antibacterial self etching system, and ozone therapy do not increase the duration of clinical treatments, they can be efficient solutions for the restorative treatment of primary teeth.

Key Words: Ozone, MDPB, Calcium hydroxide, Anti-bacterial agents.

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INTRODUCTION

Removing infected dentine is a routine protocol for the treatment of dental caries but there is no certain way to be sure if there are bacterial remains on the cavity surface or not. Even if affected dentine has removed, bacterial component may have still existed by the depth of 0.1-2.4 mm from cavity floor, through the pulp tissue.[1-4] To prevent pulp tissue from bacterial invasion which may still exist in cavity after treatment or comes from microleakage between restorative filling materials and dentine tissue; cavity cleansing systems have been considered to be used in dental practice for a long time ago.[5,6]

Different kind of treatment methods can be used for this purpose and applying calsium hydroxide [$Ca(OH)_2$] based cement on cavity floor is the oldest and most useful one of them. This material is a good choice due to its antibacterial activity and hard tissue forming capacity and thanks to that it is a gold standard for antibacterial treatment practices.[7,8]

On the other hand, Imazato[9] has developed MDPB (12-meth-acryloyloxydodecylpyridinium bromide) which is a resin monomer that has a strong antibacterial activity and doesn't effect the junctions between materials and dentine and inhibit bacterial growth.[10,11] MDPB is a compound of an antibacterial agent, quarternary ammonium with a methacryloyl group, and exhibits strong antibacterial activity against oral *Streptococci*. Among *Streptococcus* species, *Streptococcus mutants* is considered the chief etiological agent for causing dental caries. After that, this material started to be used in dentistry under the trade name of Clearfil Protect Bond.[12]

Other choice is ozone treatment that has been using in dentistry due to its antibacterial activity, new tissue forming capacity and the ability of stimulating healing. Ozone gas is the most effective anti-oxident agent in nature. It has been using due to its healing effect in medicine and there is no reported reverse effect and negative effect to bonding strengths and general health.[13] Previous studies have shown that ozonized water and ozone gas reduce the total cultivable microbiota significantly in vitro.[14-17] Baysan *et al.*[18] found a significant reduction in *S. mutans* and *Streptecoccus sobrinus* in the ozone-treated side of the root caries lesions compared with the control side.

There is a previous study in which antibacterial effectiveness of 30 seconds and 60 seconds ozone therapy applied via OzonyTronX, Clearfil Protect Bond (a MDPB containing bonding agent) and Dycal [$Ca(OH)_2$] on *S. mutans* in deciduous teeth were evaluated in vitro.[19] But there is no any other study, in which these materials are compared applying deciduous teeth in -vivo. Therefore, the aim of this study is to compare antibacterial effectiveness of CPB (contains MDPB) and ozone therapy against *S.mutans* in deciduous teeth, in-vivo.

MATERIALS AND METHODS

The study protocol was carried out according to the principles of the Helsinki Declaration, including all amendments and revisions. Collected data were only accessible to the researchers. Informed consent was obtained from all participants. Patients or their legal representatives gave their informed consent prior to any treatment of the teeth. The study was reviewed and approved by the institutional ethics board of Ankara University Faculty of Dentistry (no: 118/2; date:16.05.07).

A power analysis (Power and Precision software, Biostat, Englewood, NJ, USA) was conducted in order to determine an appropriate sample size based on previous studies. It indicated that detection of differences could be obtained with at least 10 teeth at a power of 0.8. Thus, this study was conducted using 40 deciduous teeth in total. 40 primary molars were obtained from ten patients whose ages ranged between 5 and 11 and whose all primary second molars were decayed. All decayed deciduous second molars were divided into four groups randomly according to CONSORT guideline and in that way, it could be possible to evaluate all groups in one patient's mouth (Figure 1).

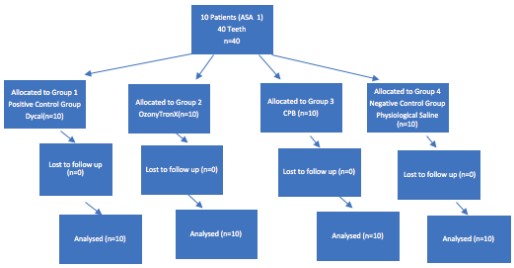

Figure 1. Consort Diagram

Group 1: Dycal (Dentsply/Caulk, Denstsply International Inc. Milford, DE, ABD) -positive control group

Group 2: OzonyTronX (Mymed, Almanya)-30 second ozone therapy.

Group 3: CPB (Clearfil Protect Bond (CPB), Kuraray, Europe).

Group 4: Physiological Saline (PS)- Negative control group.

In clinical examinations before treatment, the patients who are healthy as ASA classification (ASA 1) and whose teeth didn't have the symptoms of irreversible pulp degenerations, pathological or physiological mobility and positive response to percussion and palpation test were incorporated into study.

In radiological examinations, the criterias to select the appropriate patients for the study were; to have a healthy dentinal tissue between pulp and caries dentine and absence of external or internal root resorption. Also, surrounder bone tissue's health and condition of periodontal space and lamina dura were considered (Figure 2a, 3a, 4a).

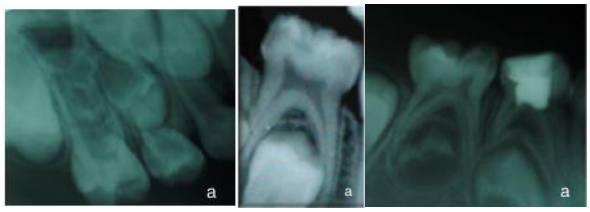

Figure 2a) The radiography of right upper deciduous second molar.
3a) The radiography of left inferior deciduous second molar.
4a) The radiography of left inferior deciduous second molar.

Treatment sessions were held in an air conditioned clinic which has only one dental unit and rubber dam was used to avoid bacterial contamination.

All teeth surfaces except caviteted areas were cleaned with savlon solutions and washed with saline solution. After that, superficial surfaces of necrotic dentin were removed with sterile steel round burs (Figure 2b, 3b, 4b).

Figure 2b) After first session, removing superficial decayed dentine tissue.
3b) After first session, removing superficial decayed dentine tissue.
4b) After first session, removing superficial decayed dentine tissue.

Cavities were prepared by a depth of 2,5 mm to make enough place for compomer restorative materials. Remained dentin was cleaned with physiological saline solution and dried with cotton pellets. For microbiological assessment, dentine samples were collected with cooled burs from the middle of cavity floor. The samples were transferred to Gazi University Faculty of Pharmacy in Reduced Transport Fluid[20] within 2 hours.

Study Groups and Control Groups:

Group 1:

Dycal was applied with sterile round handpieces on the remaining decayed dentine (Figure 2c).

Figure 2c) Applying Dycal on cavity floor.

After that, sterile sponge (VDW, Munich, Germany) was placed on the material (Figure 5) and cavity floor and the cavity were covered with

blue compomer (Twinky Star, Voco, Cuxhaven, Germany) (Figure 6a) which was polimerized with a light cure (Ultralume 5; Ultradent, S. South Jordan, UT, USA) during 20 second.

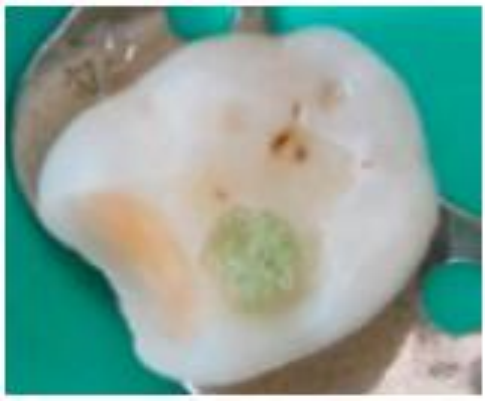

Figure 5. Placing sterile sponge on cavity floor.

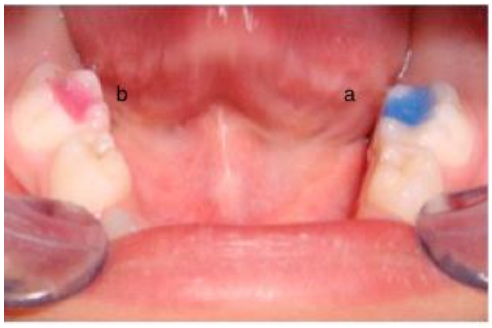

Figure 6a) Dycal

Group 2:

OzonyTronX was applied for 30 seconds in forth degree with CA Prob (Figure 3c).

Figure 3c) Performing Ozone treatment.

After that, sterile sponge and pink compomer (Twinky Star, Voco, Cuxhaven, Germany) (Figure 6b) were used to fulfill the cavity.

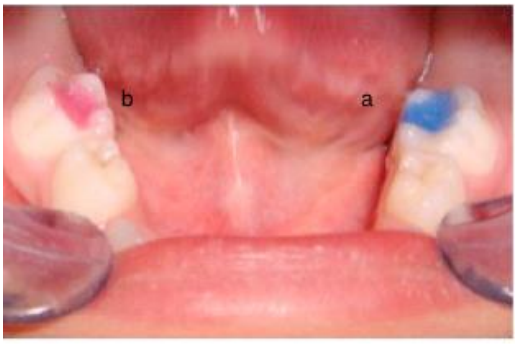

Figure 6b) Ozony TronX Group

Group 3:

CPB, which contain MDPB was applied as one drop on the remaining decayed dentin, on cavity floor (Figure 4c).

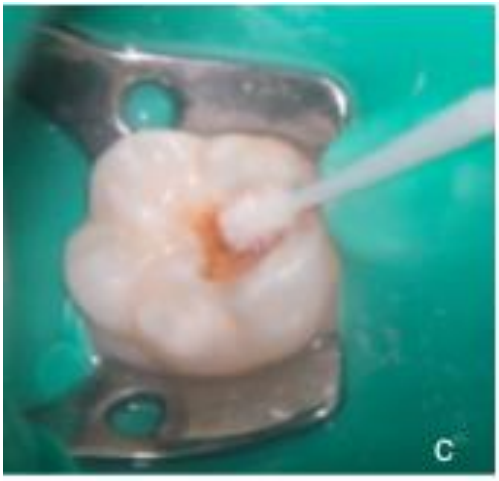

Figure 4c) Applying CPB on cavity floor.

After a period of 20 second, bonding agent was used and polymerized for 10 second. (Ultralume 5; Ultradent, S. South Jordan, UT, USA) Sterile sponge was placed on the cavity floor and green compomer (Twinky Star, Voco, Cuxhaven, Germany) was used to cover the cavity (Figure 6d).

Figure 6d) CPB Group

Group 4:

For negative control group, cavity floor was washed with P.S and sterile sponge was placed. Than, Orange compomer (Twinky Star, Voco,

Cuxhaven, Germany) was used for cavity restoration (Figure 6c).

Figure 6c) Control Group

After a period of four weeks, compomer restorations were removed from the cavities by means of a sterile diamond bur without contacting the dentine on cavity walls. Then, the dentine samples were collected for a second time by sterile steel round burs. A total CFU count was obtained through a culture (MSA plates) of dentine samples collected from each group. Counts below 20 CFU were below the limits of detection and were recorded as 0 (undetectable). All microbiological processes were carried out by a microbiologist experienced in oral microbiology for microbiological assesstments. The samples were transferred to Gazi University Faculty of Pharmacy within 2 hours.

Finally, decayed dentine was removed completely and final restorations were performed (Figure 7).

Figure 7. After second session, complete cavity cleaning.

RESULTS

The analyses of previous study's results were performed in Ankara University Faculty of Statistics by using ''Statistical Package for the Social Sciences'' software (SPSS 11.5 for windows, SPSS Inc., Chicago, Illionis, USA). Duncan Test was performed to determine the differences between groups and Kovaryans Analyses were performed before treatment. Wilcoxon test was used before and after treatment. P-values of less than 0.05 were considered to be statistically significant.

Bacterial assessment of collected tissue before treatment were shown in Table 1 and Figure 8 ($p<0.05$).

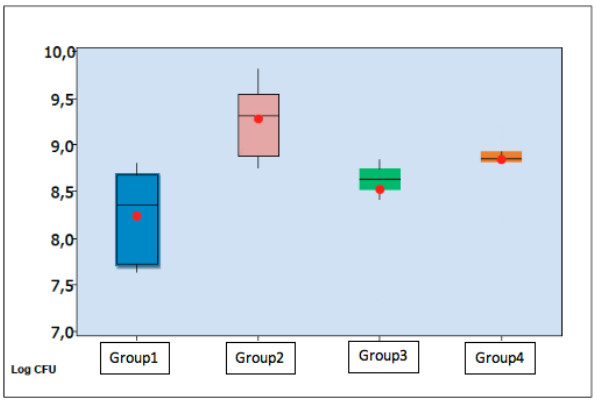

Figure 8. Microbial counts (log CFU/g dentine) in the cavites of all groups before treatment.

Table 1: Bacterial assessment (Log CFU) of collected tissue before treatment

Group	N	Mean	Median	SD	Min	Max
Group 1: Dycal	**10**	**8.23**	**8.355**	**0.465**	**7.633**	**8.799**
Group 2: OzonyTronX	**10**	**9.27**	**9.310**	**0.367**	**8.748**	**9.820**
Group 3: CPB	**10**	**8.51**	**8.633**	**0.438**	**8.322**	**8.845**
Group 4: Control	**10**	**8.83**	**8.854**	**0.261**	**8.704**	**8.904**

Differences between the antibacterial effectiveness of the materials were found to be statistically significant according to the results of the covariance analyses with randomized block design. Bacterial assessment of collected tissue after treatment were shown in Table 2 ($p<0.05$).

Table 2: Bacterial assessment (Log CFU) of collected tissue after treatment

Group	N	Mean	Median	SD	Min	Max
Group 1: Dycal	**10**	**5.463**	**5.516**	**0.222**	**5.079**	**5.699**
Group 3: CPB	**10**	**3.621**	**3.648**	**0.219**	**3.204**	**3.863**
Group 4: Control	**10**	**9.445**	**9.388**	**0.266**	**9.113**	**9.934**

Differences between group 1 were statistically significant from group 2, group 3, group 4. Group 2 was statistically significantly different from group 3 and group 4. Group 3 was statistically significantly different from group 4. Bacterial assessment of the collected tissue after treatment were Group 4 (control) > Group 1 (Dycal) > Group 3 (MDPB) > Group 2 (OzonyTronX).

After treatment, in ozone group, the bacterial caunts were below 20 CFU, below the limits of detection and were recorded as 0 (undetectable). Hence, we couldn't show the values of bacterial assessment of ozone group in the chart (Figure 9).

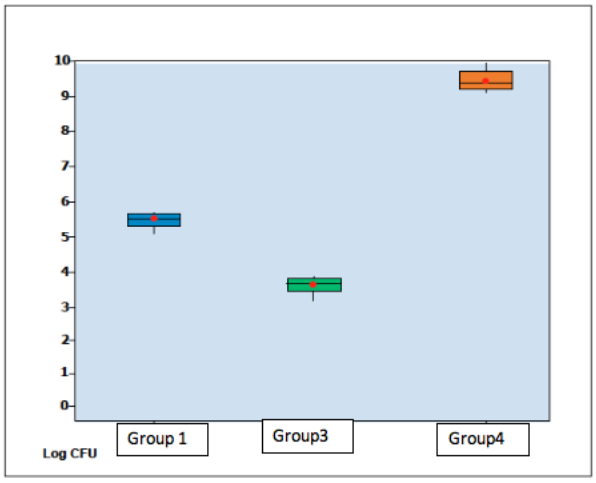

Figure 9. Microbial counts (log CFU/g dentine) in the cavites of all groups after treatment.

As result, end of the treatment, an increase was seen in the amount of microorganisms of group 4 while all the others showed decrease. Which means all the materials, except saline solution which was control group, has an effect on eliminating bacterial growth in dentinal tissue and they can be used as cavity cleansing systems in dental practices. Previous study shows that Ozone is the most effective way to protect dentinal tissue from bacterial remains.

DISCUSSION

It is always questionable if cavity preparation and removing infected dentine is a sufficient way to prevent pulp tissue from bacterial invasion or not.[21-23] To prevent the pulp tissue, lots of cleansing systems and antibacterial agents have been used and $Ca(OH)_2$ is one of them and the most acceptable one due to its antibacterial effect and remineralization activity.[24] Thus, Dycal was chosen as positive control group. Antimicrobial monomer, MDPB has been developed in recent years, which has very strong bactericidal activity against oral micro-organisms and reported against *S. mutans*.[12]

Application of ozone (OzonyTronX) has also been proposed due to its antibacterial activity.[25,26] In that study, it was aimed to evaluate the antibacterial activity of these two materials in vivo. Sterile saline was chosen as negative control group. Patients whose ages differs between 5 and 11, whose all primary molars are still exist and who is systematically healthy were chosen for the study. Thanks to that method, ıt was possible to compare all the groups in one patient's mouth. Rubber dam, which is a standard way to protect tooth from oral microflora in dental treatments, was used to avoid bacterial contamination.[27] The superficial surfaces of necrotic dentin were removed only to determine the antibacterial effectiveness of test materials.[23]

Dentin samples were collected by the burs assembled to a slowly rotating micromotor.[1] The burs were kept at -25 ^{0}C degree to eliminate the formation of heat.[28,29] Collected samples was at a quantity to fill the groves of the burs. This is also an appropriate and standard way which is used in lots of studies.[1,22,27]

Sterile sponges were applied to prevent dentin in second session. Compomer, which is most common restorative material using in pediatric dentistry was chosen as the restorative material to distinguish between groups.[30,31]

The period takes between two treatment session was decided as four weeks which is suitable for the studies that is about antibacterial activity of materials.[32] After four weeks period, patients asked about pain and sensitivity. The remaining caries were cleaned and final restorations were performed.

As a result of the study, except saline group, in all the others, there were a decrease in bacterial population in treated samples.

These results are similar to previous study which was held on cavity models in vitro. In which, a tooth cavity model was designed on cylindrical cavities created in 90 deciduous second mandibular molars. In that study, the antibacterial effectiveness of ozone therapy -60 seconds, ozone therapy -30 seconds, MDPB, MDP and $Ca(OH)_2$ were compared. *S. mutans* suspensions were inoculated in the cavities. The teeth distributed into six study group (five studying groups and one control group). Dentine samples, which were collected from the cavities before and after treatment sessions were microbiologically evaluated and antibacterial effectiveness of ozone therapy -60 seconds found statistically higher than ozone therapy-30 seconds, MDPB, MDP and $Ca(OH)_2$.[19]

Hauser-Gersparch *et al.*[33] held a study in which immediate effects of gaseous ozone and chlorhexidine gel were compared on bacteria in cavitated carious lesions in children. 30 second gaseous ozone was applied. In the end of the study, nor ozone gas neither chlorhexidine gel were found to be efficient in reducing microorganisms in open occlusal carious lesions.This results conflict with this study.

Atabek and Oztas[34] held another study to evaluate the efficiency of ozone alone and with a remineralizing solution following application on initial pit and fissure caries lesions in permanent molars. 40 second ozone gas was applied. In the end, compatibly with this study results, ozone treatment either alone or combined with a remineralizing solution was found to be effective for remineralization of initial fissure caries lesions.

CONCLUSIONS

The findings of this study indicate that ozone treatment could be considered to exert an antibacterial effect in the treatment of deciduous teeth. Conceiving that Ozone Therapy Systems could not be found easily in every dental offices, MDPB containing CPB could be a good choice for cavity disinfection process. However, further research on the long-term effects of ozone on micro-organisms, and a more detailed comparison of ozone with dentine-bonding systems and $Ca(OH)_2$, is necessary.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST STATEMENT

All authors declare no conflict of interest.

Ozon Tedavisi ve Bir Antibakteriyel Bonding Ajanın Süt Dişi Kavite Dezenfeksiyonundaki Etkinliğinin İn-Vivo Olarak Değerlendirilmesi

ÖZ

Amaç: *Bu çalışmada, OzonyTronX cihazı ile 30 s ozon tedavisi, MDPB içerikli CPB ve Ca(OH)2 içerikli bir pat olan Dycal'ın S. mutans üzerine anti bakteriyel etkinliğinin süt dişlerinde in vivo koşullarda değerlendirilmesi amaçlanmıştır.* ***Gereç ve Yöntemler:*** *Yaşları 5 ile 11 arası değişen 10 çocuk hastanın dentin çürüğü bulunan 40 adet II. süt azı dişi çalışmaya dahil edilmiştir. Tedavi öncesi toplanılan dentin örnekleri ile materyallerin uygulanmasının ardından 4 hafta sonra toplanılan dentin örnekleri mikrobiyolojik olarak değerlendirilmiş ve anti bakteriyel etkinlikleri karşılaştırılmıştır.* ***Bulgular****: Grupların S. mutans üzerinde antibakteriyel etkinliklerinin başarı sıralamaları şu şekildedir: Grup 2 (Ozon tedavisi)>*

Grup 3 (CPB)> Grup 1(Dycal). ***Sonuçlar****: Çalışma sonucunda; anti bakteriyel bir self-etching sistem olan CPB'nin ve ozon tedavisinin klinik uygulamalara ek bir süre getirmediği gibi, süt dişlerinin restoratif tedavilerinde etkin bir çözüm olabileceği kanısına varılmıştır.* ***Anahtar Sözcükler****: Anti bakteriyel etki, kalsiyum hidroksit, ozon, MDPB, kavite dezenfeksiyonu.*

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