Comparative Anti-microbial Activity of two Chelating Agent: An Invitro Study

B Rai, R Jain, S Kharb, S Anand

Citation

B Rai, R Jain, S Kharb, S Anand. *Comparative Anti-microbial Activity of two Chelating Agent: An Invitro Study*. The Internet Journal of Dental Science. 2004 Volume 3 Number 1.

Abstract

Dimercaptosuccinic acid chelating agent (DMSA) is used to remove the smear layer more effectively than EDTA. This study was conducted to determine the antimicrobial activity of DMSA and compare it with EDTA. DMSA did not show antimicrobial activity.

INTRODUCTION

The inflammatory and immunlogical reaction's in the periapical area are caused by bacteria and their toxin, immunologic agents, tissue debris and products of tissue necrosis from the pulp. Pulpal disease is the most common cause for disease of the periapical tissues. Endodontic treatment can be divided into three main phases, biomechanical preparation of root canal (cleaning, and shaping), desinfection and obturation, biomechanical preparation leads to formation of a smear layer consisting of organic and inorganic debris on the walls of canal. Smear layer formed may harbor micro-organisms as well that might cause re-infection and, it may interfere in the sealing of root canal during obturation. It is desirable to have a chemical adjunct, which removes the smear layer and possesses antimicrobial activity. Chelating agents like EDTA when used during chemico-mechanical preparation$_1$ remove the smear layer and posses anti-microbial activity, as reported by Palterson$_2$. Several investigators have shown that unless adequate irrigation is part of the canal cleaning process, debris will be left behind regardless of the irrigant used.$_3$ Ginichirohata et al. investigated oxidative potential water for it ability to remove smear layer in root canal using scanning electron microscopy. It was effective as five percent NaoCl or 17% EDTA for opening and keeping patent dentinal tubules. There is absence of toxicity in its use, and it is enough for patients to hold in oral cavity.$_4$ Karabucak et al. Compared the use of EDTA and NaoCl on the dentinal surface of root canal system after through cleaning and shaping with Ni-Ti instrumentation and observed that irrigating with 15% EDTA followed by a flush of NaoCl is most effective.$_5$ Goel et al. that five percent NaoCl solution was 80% effective in eliminating the bacterial colonies from root canal after 5th appointment whereas 15% EDTA alone was 50% effective.$_6$ Hottel et al. (1999)$_7$ reported that mesio – 2,3 Dimer Captosuccinic acid is a chelating agent which removes the smear layer when used in root canals but its anti-microbial properties have not been reported. This study was conducted in vitro to determine the anti-microbial activity of DMSA and compared with EDTA.

MATERIALS AND METHODS

Evaluation of the antimicrobial effect of the DMSA and EDTA on alpha hemoltic – streptococci (MTCC497) and staphylococcus aureus coagulase. This was done by the antibiotics sensitivity tests using.

- Disk diffusion method8,9
- Serial tube dilution method10,11

The procedures were carried out in department of microbiology, Govt. Dental College, Pt. B.D. Sharma, Post Graduate Institute of Medical Science, Rohtak (Haryana). Alpha-hemolytic streptococci (MTCC 467) and staphylococeus aureus coagulase stains were sub cultured on blood agar and mannitol salt broth respectively.

PROTOCOL FOR SERIAL DILUTION

Figure 1

Tube No	1	2	3	4	5	6	7	8	9	10	Control
BHI Nutrient Broth (ml) Chelating Agents in Serial dilution											
Discard culture Bacterial stain	Add 0.05 ml to each tube, in culture for 24 hours at 37^{0}C										

The 10% and 15% solution of meso 2,3 dimercaptosuccinic acid and ethylene diamine tetra acetic acid was prepared DMSA and $EDTA_{12}$ is insoluble in water. In order to make them soluble 10 N NaOH was used.

To prepare 10% solution, 100 mg of crystals of EDTA/DMSA were added to 500 microlitre of distilled water. The 90 microlitre solution of 10 N NaOH was then added. Distilled water was added to make 1 millilitre solution.

To prepare 15% solution, 150 mg of crystals of EDTA/DMSA were added to 500 microlitre of distilled water. The 140 microlitre solution of 10 N NaOH was then added. Distilled water was added to make 1 mililitre solution. Distilled water was added to make 1 millilitre solution. The solutions were filter sterilized using 0.65 micrometer millipore filters.

Staphylococci and streptococci were grown on mannital salt broth and blood broth for 18 hours at 37°C. One hundred microlitre at this culture was broth on respective agar plates. One hundred microlitre solution of 15% and 10% DMSA and EDTA was added in five wells (8 mm diameter).

Normal saline well act as control. In each of four test tubes five ml of mannitol salt broth was taken. Each tube was broth with one hundred microlitre of 18 hour grown culture of staphyglococcus aureus. Out of four tubes, in one tube crystals of DMSA and in second tube crystals of EDTA were added at a concentration of 1% wt/value and other two tubes were kept as control tubes. The plates and tubes were incubated at 37°C for 48 hours. Antimicrobial activity was indicated by the presence of zone of inhibition of bacterial growth around the wells containing the chemicals in agar plates. Bacterial growth was indicated in the form of turbidity in broth tubes.

OBSERVATIONS AND RESULTS

Figure 2

Table 1: Results of Serial Tube Dilution Sensitivity Test

Sr. No.	Serial dilution	1	2	3	4	5	6	7	8	9	10	Control
1	10% EDTA	-	-	-	-	+	+	+	+	+	+	+
2	15% EDTA	-	-	-	-	-	+	+	+	+	+	+
3	10% DMSA	-	-	-	-	-	-	-	-	-	-	-
4	15% DMSA	-	-	-	-	-	-	-	-	-	-	-

Figure 3

Table 2: Zones of inhibition on blood agar and mannitol agar

Sr. No.	Chelating agents	Blood agar	Mannitol agar
1	10% EDTA	+	+
2	15% EDTA	+	+
3	10% DMSA	-	-
4	15% DMSA	-	-

+ = Zone of inhibition of less than 5 mm.
- = No detechable of zone.

DISCUSSION

Bio-mechanical preparation of root canal, along with irrigation, grossly reduces the bacterial population of canal.[11] An irrigate with antimicrobial activity and which removes smear layer along with necrotic and organic debris is desirable. EDTA is a good chelating agent[1] but mesio 2-3, dimercaptosuccinic acid (DMSA) has better chelating properly than EDTA.[7]

In the study, antimicrobial activity of two chelating agent such as DMSA and EDTA were studied. EDTA showed an anti-microbial activity in concentration of 10% and 15%, broth in concentration of 1% wt/vol as previous study[2]. DMSA did not anti-microbial activity.

CONCLUSION

EDTA showed an anti-microbial act activity but DMSA did not anti-microbial activity.

CORRESPONDENCE TO

Dr. Balwant Rai S/o Sh. Ramsawroop Village – Bhangu Distt. Sirsa Post Office – Sahuwala I. E-mail : drbalwantraissct@rediffmail.com Mobile No. : 091-9812185855

References

1. Heling B, Shapiro S, Sciaky I. An in vitro comparison of amount of calcium removed by sodium salt EDTA and hydrochloric acid during endodontic procedures. Oral Surg 1965; 19: 531-533.
2. Patterson S.S. In vivo and in vitro studies of the effect of the disodium salt of ethylenediamine tetra acetate on human dentine and its endodontic implications. Oral Surg 1963; 16: 83-103.
3. Louis I, Grossman, Oliet S, Carlos E. Delrio. Endodontic practice : in preparation of root canal: equipment and technique for cleaning, shaping and irrigation. Var ghese Publishing House: Bombay (11 ed.) Indian Edition 1988; pp. 179-188.
4. Ginichirohata, Manbu, Franklin SW et al. Removal of smear layer in the root canal using oxidative potential water: Journal of Endodontic 1996; 22(12): 212-216.
5. Karabucak B, Wong R, Pypen C. SEM study of different irrigating solution's on dentinal walls after Nickel - titanium rotary instrumentation : Journal of Endodontics 1997; 23(4): 251.
6. Goel M, Loomba K et al. An in vivo evaluation of anti-bacterial effect of irrigating solution in endodontics : Journal

of Indian Endodontic Society 1989; 2: 12-14.
7. Hottel TL, EI - Refai NV, Jones JJ. A comparison of the effect of three chelating agents on the root canals of extracted human teeth : Journal of Endodontic 1999; 25: 716-717.
8. Gradwahl's. Clinical laboratory methods and diagnosis. 8th Indian Edition. BI Publications Ltd. 1990; 2: pp. 1941-46.
9. Howard BJ. Clinical and pathologic microbiology: st. Louis. Mosby (2nd Edi.); pp. 161-66.
10. Mackie and McCartney. Practical medical microbiology. Churchill Livingstone (13th Edi.) 1989; pp. 173-174.
11. Myers RM, Koshi G. Diagnostic procedures in medical and immunology / serology, microbiology laboratories. Christian Medical College and Hospital, Vellore, India - Revised 1982; pp. 86-88.
12. Seidborg BH, Schiloler H. An evaluation of EDTA in endodontic. Oral Surg 1974; 37: 609-620.
13. Strindberg L. Intracanal medication endodontics. In Ingle: Phiadelphia (4th Edi.) 1994; p. 427.

Author Information

Balwant Rai, B.D.S. Intern
Government Dental College, Pt. Bhagwat Dayal Sharma, Post Graduate Institute of Medical Science

Rajnish Jain, (M.D.S.) Endodontics
Government Dental College, Pt. Bhagwat Dayal Sharma, Post Graduate Institute of Medical Science

Simmi Kharb, (M.D.) Bio-chemistry
Pt. Bhagwat Dayal Sharma, Post Graduate Institute of Medical Science

S. C. Anand, (M.D.S.) Oral & Maxillofacial Surgery and Orthodontics
Government Dental College, Pt. Bhagwat Dayal Sharma, Post Graduate Institute of Medical Science