10.5005/jp-journals-10024-1976

ORIGINAL RESEARCH

Antibacterial Activity of Two Chemomechanical Caries Removal Gels on Carious Dentin of Primary Teeth: An *in vitro* Study

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ABSTRACT

Introduction: Chemomechanical caries removal (CMCR) is an effective method of caries removal especially for primary teeth as they cause less discomfort when compared with conventional caries removal. The most significant thing about caries removal is the elimination of cariogenic bacteria. This study compares the antibacterial activity of two CMCR gels.

Materials and methods: A total of 40 primary molar teeth with carious dentin were split along the long axis in a laboratory. Total viable count (TVC) was taken for the teeth before splitting as a measure of colony-forming units per milliliter (CFU/mL). Each half was treated with either Carisolv or Carie-Care CMCR gels. Clean dentin samples were evaluated for *Streptococcus mutans* (SM) and *Lactobacillus acidophilus* (LB) after removal of carious tissue using the caries removal gels using serial dilutions and incubating on specific agar plates.

Results: The results showed significant reduction in mean TVC after use of both the CMCR gels. Both gels reduced the CFU/mL of SM and LB to a significant level ($p < 0.05$). However, there was no significant difference between the antibacterial activities of the two CMCR gels.

Conclusion: The CMCR gels (Carisolv and Carie-Care) significantly reduced the residual TVC as well as SM and LB in carious primary dentin. Both CMCR gels had a similar antibacterial activity on the carious dentin of primary teeth.

Clinical significance: The CMCR gels tested have a significant antibacterial activity and can be effectively used for elimination of caries-causing bacteria in primary teeth.

Keywords: Antibacterial activity, Carie-Care, Carisolv, Chemomechanical caries removal, Primary teeth.

How to cite this article: Gulzar S, Arora R, Shah AH, Bhardwaj B, Abusalim G, Khalil HS, Wyne AH. Antibacterial Activity of Two Chemomechanical Caries Removal Gels on Carious Dentin of Primary Teeth: An *in vitro* Study. J Contemp Dent Pract 2016;17(12):1027-1032.

Source of support: Nil

Conflict of interest: None

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INTRODUCTION

A better understanding of the caries process and the potential for tooth remineralization has reversed the philosophy of GV Black's principle of "extension for prevention" to "prevention of extension," the prime goal being the preservation of natural tooth structure.[1] Dental caries is now seen more as a bacterial infection rather than just a mechanical defect in the tooth.[2] Mount and Ngo advocated that restoration of teeth *per se* will not prevent or eliminate dental caries unless the cariogenic

bacteria are eliminated. They further advocated that the "minimally invasive dentistry" concepts depend on the demineralization–remineralization cycle, adhesion, and biomimetic restorative materials.[3]

It has been advocated that there is a need to develop minimally invasive methods for caries removal, cavity preparation procedures, and to increase patient comfort.[4] Conventional methods of caries removal are considered painful and unpleasant, especially for children due to use of local anesthesia to control discomfort.[5] There are several alternatives to conventional caries removal and cavity preparation including air abrasion; air polishing; ultrasonic instrumentation; sonoabrasion; heal ozone; laser techniques; and chemomechanical caries removal (CMCR) methods. The common goal of all these techniques is their attempt to remove carious dentin only, thereby avoiding the painful and excessive preparation of sound tooth structure. However, none of these caries removal methods seems to be ideal.[6] Nevertheless, CMCR method is efficient in removing infected dentin without altering the healthy dentinal tissue.[7]

Several chemical compositions have been used for CMCR, such as GK-101, Caridex (MediTeam Dental AB, Stockholm, Sweden), and various enzymes. Efforts to develop an ideal CMCR agent resulted in a newer agent: Carisolv (MediTeam AB, Göteborg, Sweden).[8] It claims to remove infected, degraded, and demineralized dentin only, leaving the unaffected dentin layer behind.[9] It also possesses an antibacterial effect on the carious dentin of primary teeth.[10] Carisolv has been shown to be biocompatible with the dental pulp and exhibits hemostatic properties as well.[11] Other CMCR gels are papain-based compounds[12] and have been effectively used for caries removal in children.[13] Moreover, CMCR methods seem to be beneficial for caries removal in children and patients with dental anxiety, aiding to avoid the discomfort caused by a conventional caries removal method.[13,14]

Carie-Care (Biosynergetics, India) is another CMCR gel that has been locally introduced. Its active ingredients are derived from papaya extract. This gel preparation does not contain sodium hypochlorite or any other strong chlorinating agent, instead has most of the ingredients derived from natural sources.[15]

Although several studies have compared CMCR in terms of patient's comfort, clinical time required, caries removal and effect on sound dentin and pulp:[16,17] yet, very few studies have compared the antibacterial activity of the CMCR gels.[18] The present study was aimed at comparing the antibacterial activity of two CMCR gels: Carisolv and Carie-Care. More specifically, the effect of the CMCR gels was observed for the caries-causing bacteria *Streptococcus mutans* (SM) and *Lactobacillus acidophilus* (LB).

MATERIALS AND METHODS

The study was an *in vitro* study conducted in the laboratory at the Department of Microbiology, Regional Research Centre, Udaipur, Rajasthan. The extracted primary teeth required for the study were collected from the Department of Pedodontics and Preventive Dentistry, Darshan Dental College and Hospital, Udaipur.

Materials

Carisolv™ gel (Mediteam, Sweden)

It is marketed as a two-gel system. The gel #1 contains glutamic acid, leucine, lysine, sodium chloride, carboxymethylcellulose, water, and sodium hydroxide at a pH of 11. The other gel contains 0.5% sodium hypochloride and alanine aminotransferase.[19]

Carie-Care gel (Biosynergetics, India)

This product that has been locally introduced has its main active ingredient from papaya extract, an endoprotein, chloramines, and dye. In addition, the preparation contains specific percentages of essential oils from plant sources, which have anti-inflammatory and mild anesthetic effect.[15]

METHODOLOGY

Forty extracted primary molar teeth (Table 1) were cleaned and handled according to infection control protocols set by the Centers for Disease Control (CDC); these teeth were stored in sterile phosphate-buffered saline (HiMedia Ind) using sterile-capped test tubes and quickly processed for microbiological procedures.

Each tooth was split in two halves in a laminar flow chamber using a diamond cutting disk fixed in a straight handpiece. Samples for carious dentin of the teeth were then cultured into 10 mL agar plates by serial dilutions to determine the colony-forming units per milliliter (CFU/mL). Schaedler agar was used to determine the total viable count (TVC). For streptococci isolation, Mitis Salivarius Agar was used and for *Lactobacillus* species isolation, the MRS agar was used. All experiments were done in triplicates. Three experimental groups were formed for analysis.

Group I: This group consisted of carious dentin sample from extracted teeth. A sample of the carious dentin was taken with a sharp spoon excavator prior to splitting (sample 1).

Group II: Each tooth was split into two portions along its long axis; one half of each carious tooth was treated with Carisolv and sample of apparently clean dentin (sample 2) was collected using the instrument provided with the Carisolv pack.

Table 1: Distribution of the sample teeth along with the carious lesion

	Sample teeth				
Site of caries	*Primary lower first molar*	*Primary lower second molar*	*Primary upper first molar*	*Primary upper second molar*	*Total*
Occlusal	9	4	4	4	21
Mesial	3	3	2	1	9
Distal	1	2	1	0	4
Mesio-occlusal	0	1	1	0	2
Disto-occlusal	1	0	0	1	2
Mesio-occluso-distal (MOD)	2	0	0	0	2
Total	16	10	8	6	40

Group III: Other half of each carious tooth was treated with Carie-Care and sample of apparently clean dentin (sample 3) was collected using a sharp spoon excavator.

The CFU/mL for each group was determined using serial dilutions plated on the specific agar plates. The agar plates were incubated anaerobically at 35°C for 48 hours. *Streptococcus mutans* and LB in all samples were identified using the specific agar plates (HiMedia Ind).

Table 2: Reduction in total mean viable count of the bacteria after use of both the chemomechanical gels

Reduction in TVC of bacteria (mean) CFU/mL			
	Mean viable count	*Std. Dev.*	*$Sig (2 tailed)*
Total viable count (TVC)	85.09×10^4	5.806	
TVC after Carisolv app	19.68×10^4	2.269	0.007*
TVC after Carie-Care app	20.45×10^4	3.178	0.000*

*Significant at $p < 0.05$; $Mann–Whitney U test

Statistical Analysis

The data obtained were entered into a computer. Statistical Package for Social Sciences (SPSS) (version #20) was used to enter and analyze the data. The data were tabulated and statistical analyses were conducted using Wilcoxon signed rank test and Mann–Whitney U test. Pair-wise comparison of mean values was done using Wilcoxon signed rank test, while the Mann–Whitney U test was applied to find out the difference between the two independent groups.

RESULTS

An *in vitro* study was conducted to evaluate and compare the antibacterial activity of two commercially available caries removal gels, i.e., Carisolv and Carie-Care, on carious dentin of 40 primary molar teeth (Table 1).

Table 2 shows reduction in mean TVC after use of both the chemomechanical gels. Mean TVC of 40 carious dentin sample was 85.09×10^4, which was reduced to 19.68×10^4 after application of Carisolv ($p = 0.007$) and to 20.45×10^4 with the application of Carie-Care gel ($p = 0.000$). Hence, a statistically significant reduction in the TVC was achieved with both the CMCR gel systems tested.

Table 3 shows effect of CMCR gels on *S. mutans* and LB CFU/mL after application of each gel. Mean CFU/mL of *S. mutans* for 40 untreated sample was 6.98×10^4 before the application of the gels. It was reduced to 0.68×10^4 after Carisolv application ($p = 0.000$), and to 0.80×10^4 after Carie-Care application ($p = 0.000$). Similarly, for LB, the untreated colony count mean was 6.13×10^4 reduced equally by Carisolv and Carie-Care (mean CFU/mL 1.03×10^4).

Table 4 shows comparison of TVC and reduction in CFU/mL of *S. mutans* and LB after the application of Carisolv and Carie-Care. No significant differences between the two tested CMCR systems were found. Although Carisolv showed slightly higher antibacterial activity than Carie-Care for both *S. mutans* and LB CFU/mL, the difference was not statistically significant (Graph 1).

Table 3: Effect of caries removal gels on *Streptococcus mutans* and *Lactobacillus* colony forming units (CFU/mL) after application of each gel

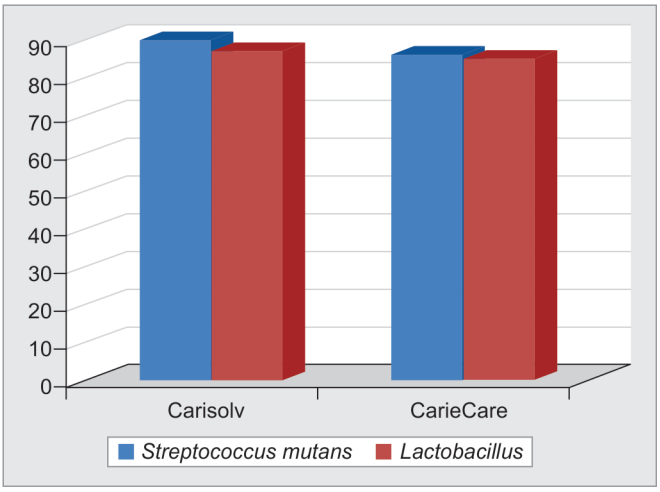
	Mean	*Std. Dev.*	*Std. Error Mean*	*$Sig*
Streptococcus mutans				
CFU for Strep untreated	6.98×10^4	2.626	0.415	
CFU for Strep after Carisolv app	0.68×10^4	0.917	0.145	0.000*
CFU for Strep after Carie-Care app	0.80×10^4	0.911	0.144	0.000*
Lactobacillus				
CFU for *Lactobacillus* untreated	6.13×10^4	3.180	0.503	
CFU for *Lactobacillus* treated with Carisolv	1.03×10^4	1.187	0.188	0.000*
CFU for *Lactobacillus* treated with Carie-Care	1.03×10^4	1.291	0.204	0.000*

*Significant at $p < 0.05$; $Mann–Whitney U test

Table 4: Comparison of TVC, reduction in CFU/mL of *Streptococcus mutans* and *Lactobacillus* after the application of Carisolv and Carie-Care

	Paired samples test							
	Paired differences							
				95% confidence interval of the difference				
	Mean	Std. Dev.	Std. error mean	Lower	Upper	t	df	$Sig. (2-tailed)
TVC after Carisolv app *vs* TVC after Carie-Care app	0.775	3.068	0.485	–1.756	0.206	–1.598	39	0.118
CFU for SM after Carisolv app *vs* CFU for SM after Carie-Care app	0.125	1.223	0.193	–0.516	0.266	–0.646	39	0.522
CFU for LB treated by Carisolv *vs* CFU for LB treated by Carie-Care	0.000	1.109	0.175	–0.355	0.355	0.000	39	1.000

$Wilcoxon signed rank test; SM: *Streptococcus mutans*; LB: *Lactobacillus acidophilus*



Graph 1: Percentage reduction in *Streptococcus mutans* and *Lactobacillus* after application of Carisolv and Carie-Care

DISCUSSION

The purpose of this *in vitro* study was to compare the antimicrobial effects of two CMCR gels, Carisolv and Carie-Care. Using similar methodology, Draghincescu[19] compared the effect of Carisolv and conventional caries removal with a handpiece bur on cariogenic bacteria. The results showed that caries treatment by the Carisolv-2 system was more efficient than drilling; and the Carisolv-2 gel had antibacterial effect. In the present study, difference in the bacterial count before and after application of CMCR agents was considered as the measure of antibacterial efficacy. El-Tekeya et al[20] carried out a similar study on effectiveness of two CMCR agents, Carisolv and Papacarie, on dentin of primary teeth. They concluded that both CMCR gels reduced cariogenic bacteria significantly. However, they found Papacarie is significantly more efficient in reducing the residual cariogenic bacteria in the dentin of primary teeth *vs* both Carisolv and the hand excavation method.

Chemomechanical caries removal combines atraumatic characteristic with bactericidal and bacteriostatic actions.[21] Results of a CMCR study for children show that chemomechanical treatment of carious dentin could be as effective as traditional caries removal technique, causes less pain, and lowers the need for local anesthesia.[22] Current literature reports that Carisolv is the most commonly used chemomechanical product to remove dental caries.[23] Although many studies have proven the efficiency of Carisolv in caries removal,[5,7,9] there are reports that it did not completely remove the dental caries and, a handpiece was needed to complete caries removal.[23]

Bacteriological analysis was the method of assessment chosen in this study to test the effectiveness of both CMCR gels (Carisolv and Carie-Care) for caries removal. A reduction in the cariogenic bacterial counts was assessed after the application of the two gels, as the presence of bacteria has been considered by investigators to be an accurate indicator of infected carious dentin.[24,25] Since the number of microorganisms isolated from a site can be influenced by the method of sample collection, a reliable and standardized method was utilized in the present study. Banerjee et al[6] used a standardized procedure for obtaining a dentin sample by means of a round bur of a defined size to establish reproducibility. In the present study, the dentin samples were carefully removed with sterile sharp spoon excavators of same diameter, both for Carisolv and Carie-Care, to avoid bias in the sample collection. This method was preferred as it reduces loss of sample material that may occur with the bur method. The bur may spread the dentin particles during its rotation; therefore, more cutting of sound dentin may be needed. Moreover, the dentin particles can be easily visualized on the excavator rather than the blades of the bur. The sampling method used in this study was similar to that of Azrak et al.[24] The cultures in this study were processed anaerobically. The composition of microflora changes as the lesion progresses, and obligate anaerobes become the predominant cultivable bacteria.[26] In this study, complete caries removal was assessed by the most widely used visual and tactile clinical criteria, described by Ericson et al,[27] in which there is

no tug-back sensation or catch of the probe when passed over the caries-free dentin.

In this study, the carious dentin samples that were taken before the application of CMCR agents contained higher TVC of bacteria. This is in agreement with a similar study conducted by Azrak et al.[24] In the samples taken following the application of CMCR agents, the TVCs were significantly lower. Other bacteriological studies have reported similar results.[24,25] This antibacterial effect, however, is significantly helped by mechanical removal of the infected carious dentin.[28]

In our study, carious dentin showed significantly higher CFU/mL for *S. mutans* and LB than noncarious dentin. Similar findings have also been reported by other researchers.[24,28-30] Noncarious dentin treated with either caries removal gels demonstrated significantly lower bacterial counts. This indicates that both gels have *in situ* antibacterial effect. This is in accordance with results from Carisolv-1 studies in adults[28] and in children.[24] Lager et al[28] attributed the bacterial reduction in Carisolv-treated cavities to the antibacterial properties of Carisolv, which contains chloramines that have an inherently antibacterial effect, in addition to sodium hypochlorite. Both of these substances have biocidal properties and are used as disinfectants.

Studies done by El-Tekeya et al[20] and Ammari et al[21] showed that Carisolv significantly reduced the *S. mutans* to a greater extent than that of LB. This might be due to the fact that *S. mutans* are thought to be caries-initiating bacteria, while LB are thought to be the one that predominates in deep cavities.[31] In the present study, although Carisolv reduced greater percentage of *S. mutans* than LB, the difference was not statistically significant. Overall, Carie-Care reduced lesser percentage of both *S. mutans* and LB when compared with Carisolv, again the difference was not statistically significant.

In the current study, both CMCR caries removal methods (Carisolv and Carie-Care) were efficient in reducing or eliminating microbial flora from the tooth cavity, with no superiority over each other. Residual bacteria were found in the apparently clear dentin. The clinical importance of infected dentin remaining after carious treatment is still not clear. While some researchers have suggested bacteria remaining after a caries removal procedure can be considered a clinically significant risk for further carious progression,[20,32,33] others have reported that about 102 CFU/mL of remaining bacteria in dentin from the cavity floor is considered clinically insignificant.[30] The small amount of residual microorganisms could be negligible, as it does not exceed the clinically accepted level.[33,34] Moreover, with the new adhesive restorations providing completely sealed margins and with the recently introduced antimicrobial cavity cleaners, this small amount of bacteria would seem to have a trifling effect on producing any further demineralization.[20,35]

The results of this study have important implications in the management of dental caries in children. Although Carisolv was slightly more efficient in reducing the residual cariogenic bacteria than Carie-Care, both CMCR gels seem to effectively remove carious dentin. To mention limitations of the present study, a bigger sample size might have yielded more reliable results. Also, the time consumed in actual microbiological evaluation and the sectioning of the teeth might affect the bacterial counts. In addition, actual oral environment cannot be produced through an *in vitro* study.

CONCLUSION

The CMCR gels (Carisolv and Carie-Care) significantly reduced the residual TVC of mutans streptococci and lactobacilli in carious primary dentin. Both CMCR gels had a similar antibacterial activity on the carious dentin of primary teeth.

ACKNOWLEDGMENT

The authors would like to acknowledge the support of all the personnel working in the laboratory at the Department of Microbiology, Regional Research Centre, Udaipur, Rajasthan, India. The authors would also like to specifically mention the support provided by Dr Savita K Meena in helping out with the microbiological laboratory procedures.

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