THE EFFECT OF PROBIOTIC MOUTHRINSE ON PLAQUE AND GINGIVAL INFLAMMATION

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Original Article

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ABSTRACT

This study evaluated the effect of a Probiotic mouthrinse containing nisin, a bacteriocin extracted from *Lactococcus lactis* on dental plaque and gingivitis in young adult population.

A group of 32 subjects were randomly assigned into two groups of 16 each. The first group started using the control mouthrinse (placebo) for 2 weeks followed by a washout period of 4 weeks. This group then used the test mouthrinse (Probiotic) for a further duration of 2 weeks. The second group followed a similar protocol as the first except that this group started with the test mouthrinse (Probiotic). Plaque Index (PI) and Gingival Index (GI) were recorded at baseline and after 2 weeks for each group. All subjects were given full mouth prophylaxis after each measurements.

The results of this study showed that rinsing with Probiotic mouthrinse resulted in a statistically significant reduction of plaque accumulation and gingivitis compared to rinsing with placebo. The results indicated that Probiotic mouthrinse containing nisin had the potential of inhibiting plaque accumulation and was effective in reducing gingivitis.

Key word: probiotic; mouthrinse; plaque; gingivitis

INTRODUCTION

The primary cause of periodontal disease is bacterial irritation. Dental plaque accumulations are the prerequisite for the development of gingivitis (1). Current opinion favours the concept that plaque-induced gingivitis always precedes periodontitis (2) although not all gingivitis proceed to periodontitis (3).

The long term success of periodontal treatment is dependent on satisfactory oral hygiene practices by individuals to maintain plaque levels compatible with gingival health (4). Periodontal treatment is also directed towards eliminating subgingival plaque which itself is derived from supragingival plaque.

Supragingival plaque control is thus fundamental to the prevention and management of periodontal disease and with appropriate advice and instructions from professionals, is primarily the responsibility of the individual, using tooth brushes and interdental cleaning (5). Unfortunately, it is a fact of life that a significant proportion of individuals fail to practice a high enough standard of plaque removal thus gingivitis is highly prevalent from an early age (6, 7).

Therefore, chemical agents have increasingly been used as adjuncts to mechanical plaque control. They are intended to augment, not to replace, mechanical plaque control (8). It is now recognized that chemical anti-plaque agents may be of value at inhibiting or reducing plaque formation and thus gingival inflammation (7, 9).

The most tested and effective anti-microbial agent known today is chlorhexidine which has been used for more than two decades (10). Chlorhexidine digluconate is, to date, the thoroughly studied and the most effective anti-plaque and anti-gingivitis agent (11). In oral use as a mouthrinse, chlorhexidine has been reported to have a number of local side effects. These side effects are brown discolouration of the teeth and tongue, oral mucosal erosion and taste perturbation (12). Several side-effects associated with its use have stimulated the search for new chlorhexidine solutions with various concentrations and flavoring agents, as well as alternative anti-plaque agents (13).

A Probiotic mouthrinse contains nisin, bacteriocins of short chain polypeptides produced by *Lactococcus lactis* cultured in a fermentor. These peptides are separated and purified from all other components including the lactic acid bacterial cells and then incorporated into the mouthrinse.

Bacteriocins are one of a number of antimicrobial substances produced by lactic acid bacteria (LAB), including organic acids, hydrogen peroxide, diacetyl and inhibitory enzymes (14,15). Bacteriocins are loosely defined as biologically active protein moieties with a bacteriocidal mode of action (16, 17). Bacteriocins are ribosomally synthesized peptides or proteins and usually act against closely

related species. Since bacteriocins are odorless, colorless and non-toxic, they adhere to the requirements set out for food preservatives (18).

Bacteriocins differ from traditional antibiotics in one critical way: They have a relatively narrow killing spectrum and are only toxic to bacteria closely related to the producing strain. These toxins have been found in all major lineages of bacteria and more recently, have been described as universally produced by some members of the Archaea (19).

The prototype LAB bacteriocin, nisin, was first discovered in 1928, when Rogers L.A. observed metabolites of *Streptococcus lactis* (now reclassified as *Lactococcus lactis*) were inhibitory to other LAB. The commercial application of nisin is in the preservation of a number of processed foods, and the award of FDA approval in 1988 for its use as a biopreservative elicited considerable interest in other bacteriocins from GRAS (generally regarded as safe) organisms. Commercial nisin is produced by large-scale fermentation of media containing food-grade ingredients.

Nisin is bactericidal against a wide range of Gram-positive bacteria (20). Nisin has been shown to kill a wide range of organisms including *Actinomyces, Bacillus, Clostridium, Corynebacterium, Enterococcus, Gardnerella, Lactococcus, Listeria, Micrococcus, Mycobacterium, Propionibacterium, Streptococcus,* and *Staphylococcus* (21). It is also active against Gram-negative bacteria such as *Camphylobacter, Haemophilus, Helicobacter, Neisseria, Escherichia coli and Salmonella* species provided that the outer membrane is damaged thus nisin is used in combination with other compounds such as chelating agents (22).

Howell et. al, (23) conducted a study to evaluate the effect of a mouthrinse containing nisin on the development of plaque and gingivitis in beagle dogs when compared to 0.12% chlorhexidine and placebo. The author concluded that nisin was an antimicrobial agent and was effective in the reducing plaque build-up and gingivitis in the beagle dogs. No similar study had been conducted in human.

Turner et. al. (24) conducted an in-vitro investigation of the antibacterial effect of nisin in root canals and canal wall radicular dentin. The aim of the study was to determine the efficacy of nisin (bacteriocin) in killing *Enterococcus faecalis* and *Streptococcus gordonii* within the root canal system. The author concluded that nisin was effective at eradicating *Enterococcus faecalis* and *Streptococcus gordonii* cells in pure culture and was comparable to $Ca(OH)_2$ in the elimination of these species from within the root canal system.

Johnson et. al, (25) conducted a study to determine the effects of nisin on the microbial flora of dental plaque of monkeys (*Macaca fascicularis*). In conclusion, there were fewer streptococci in the plaque of the dogs that received nisin in their foods. The finding that streptococci formed a lesser proportion of the plaque flora was associated with a concomitant increase in the proportion of other bacteria but the increase was not significant. This suggested that nisin did not alter the eco-system of micro-flora in the oral cavity.

Nisin has never been used as an ingredient in any mouthrinse and thus there was no information on its efficacy. The aim of this study is therefore, to compare plaque accumulation and gingival inflammation in subjects who used mouthrinse containing probiotics and placebo.

MATERIAL AND METHODS

A group of 32 subjects were recruited from patients who came for treatment in the Dental Faculty, University Malaya. Prior to their participation, they were screened to ascertain if they conformed to the criteria for the study. They were free from systemic disorders, were non-smokers and not on medications or antibiotics in the last 6 months. They were given written and verbal explanations and instructions pertaining to the study. Consent forms were signed by all participants.

The trial design is a placebo-control, double-blind and crossover type consisting of two 14-day test periods separated by a washout period of 4 weeks. During the entire study, the participants continued to exercise their regular non-supervised, self-performed oral hygiene measures. They were provided with a standardised toothbrush and toothpaste.

The participants were assigned into two groups, those receiving the mouth rinse in the order active/ placebo (group A) and those in the order placebo/ active (Group B). The mouth rinse was dispensed through a staff of the department who held a sealed code-breaker. Due to the double-blind design, all solutions had similar colour and were kept in the same kind of bottles.

All subjects were rendered scaling and polishing prior to commencement of the study. This was because some of the subjects had had dental check-up with scaling done only recently whereas some others had not had scaling for the past few years. A period of two weeks was then allowed to elapse before the first test period to obtain more a standardized levels of oral hygiene.

Baseline measurements of Plaque Index (PI) (Turesky et al., 1970) and Gingival Index (GI) (Löe and Silness, 1963) were taken from all the participants after full mouth prophylaxis were carried out. All patients will have a baseline PI of 0. They were instructed to continue with their routine tooth brushing methods. The designated mouthrinses were dispensed according to the groups of the subjects. Group A started with the active

product whereas Group B with the placebo. They were instructed to rinse twice daily about 30 minutes after tooth brushing with 15 ml of the solution for 60 seconds, followed by expectoration of the residual mouthrinse. On day 14, all subjects returned for clinical measurements. This was followed by scaling and polishing of all teeth again.

An interval of four weeks was given after the first test period so that the effects from the previous mouthrinse did not carry over into the next test period. During the washout period the subjects continued to exercise toothbrushing but refrained from using any mouthrinse.

The measurements for PI and GI were repeated after prophylaxis (PI = 0). The subjects were allotted the alternative mouthrinse and instructed to use it exactly as they had with the previous product. They returned two weeks later for clinical measurements. All subjects were then offered full mouth prophylaxis.

STATISTICAL ANALYSIS

The results were analyzed using the Statistical Package for Social Science System (SPSS) Version 12.0. For comparison between test (Probiotic) and control (placebo) groups, Paired Sample t-test was employed where the significance level was $p<0.05$. General Linear Model was used to conduct the test within-subjects effects for PI and GI which was computed using alpha = 0.05.

RESULT

The mean PI values for both mouthrinses were 0 at baseline as scaling and polishing was done for all tooth surfaces (Figure 1). A plaque index score of 0 represented a tooth surface that was entirely free of clinically detectable plaque. At the day 14 examination, when comparison to the baseline data was made, there was a significant increase in mean PI scores of placebo rinse compared to probiotic rinse ($p< 0.001$). The mean PI scores after Probiotic rinse was 0.24 ± 0.08, which was lower as compared to mean PI scores after placebo rinse which was 0.37 ± 0.12 (Figure 1). The degree of increment of mean PI scores was more pronounced for the placebo rinse compared to Probiotic rinse. Probiotic rinse caused less plaque accumulations than placebo rinse. Tests of within-subjects effects for PI for the Probiotic and Placebo showed the p-value < 0.001. Thus, the difference in the mean PI scores before and after rinse for Probiotic and placebo mouthrinse was significant.

The mean GI value for Probiotic mouthrinse at baseline was similar as compared to mean GI value for placebo (Figure 2). At the day 14 examination, in comparison to the baseline data, there was a significant decrease in mean GI scores of Probiotic rinse compared to placebo rinse ($p< 0.001$). The mean GI scores after Probiotic rinse was 0.69 ± 0.29, which was lower as compared to mean GI scores after placebo rinse which was 0.91 ± 0.26. The degree of reduction of mean GI scores was more pronounced for the Probiotic rinse compared to placebo rinse (Figure 2). Tests of within-subjects effects for GI for the Probiotic showed the p-value < 0.001. Thus, the difference in the mean GI scores before and after rinse for Probiotic mouthrinse was significant. For placebo, although there was a reduction in gingivitis, the difference was not significant ($p>0.05$).

DISCUSSION

The purpose of this study was to compare plaque accumulation and gingival inflammation between placebo mouthrinse and Probiotic mouthrinse which contained nisin, a bacteriocin extracted from *Lactococcus lactis*. This 14-day crossover study was to evaluate the efficacy of Probiotic mouthrinse as an adjunct to conventional mechanical oral hygiene procedures.

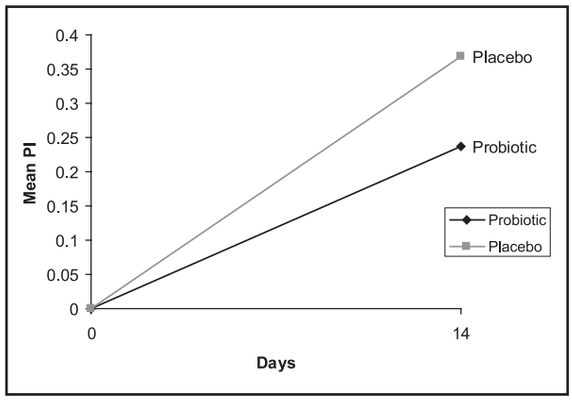

Figure 1: Line chart of mean PI scores at day 0 and at day 14 for Probiotic and placebo group.

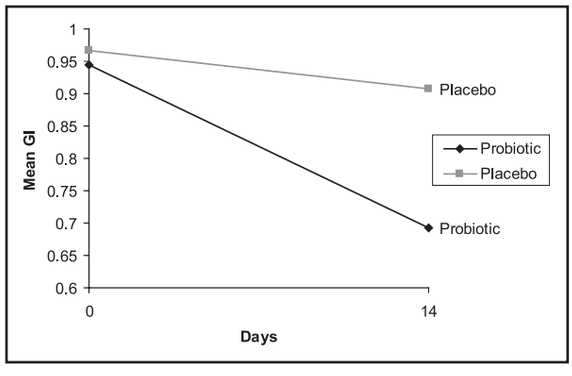

Figure 2: Line chart of mean GI scores at day 0 and day 14 for Probiotic and placebo group.

In the crossover design, each participant receives each of the treatments in a randomized order. This design enables a substantial increase in power compared to parallel group studies. The drawback to within-subject designs is contamination or carryover. In many crossover studies, it is perfectly possible that some effect of the treatment used in one period might persist and alter the response observed in later treatment periods. So, to overcome this problem in a cross over study, a washout period of carefully chosen duration between successive treatment periods is incorporated. In the present study, a washout period of 28 days was allowed between each treatment period. This was deemed sufficient when evaluating non-chlorhexidine containing agents or products to allow the oral environment to return to its original status (26).

In this study, there was a significant difference found between mean PI and mean GI between Probiotic rinse and placebo rinse after 14 days in comparison to the baseline ($p< 0.001$). The Probiotic rinse produced a 13.2% difference in mean PI scores compared to placebo rinse i.e. Probiotic rinse caused less plaque accumulations than placebo rinse. The Probiotic mouthrinse also produced a gingivitis reduction of 25.2% on day 14 compared to day 1, whereas the placebo mouthrinse produced only 5.8% reduction on day 14 compared to day 1. Therefore, the findings of this study showed that Probiotic mouthrinse had potential therapeutic value in the prevention of plaque formation and reducing gingivitis.

From this study, it was observed that Probiotic mouthrinse had a significant inhibitory effect on plaque accumulation and gingivitis for the test groups. Thus, it can be proposed that Probiotic mouthrinse had a potential therapeutic value in the prevention of plaque formation and reducing gingivitis. Its anti-plaque activity may be achieved in various ways; these include reducing the adhesion of bacteria to the tooth surface, inhibiting the growth and proliferation of microorganisms on the tooth surface, inhibiting the formation of the intercellular plaque matrix, modifying plaque biochemistry to reduce the formation of cytotoxic product and modifying plaque ecology to a less pathogenic flora (27). It was also shown by earlier researcher (20) that nisin, the active ingredient in Probiotic mouthrinse had bactericidal activity against a wide range of Gram-positive bacteria. Experimental studies on plaque development had shown that the early colonizers are streptococci, particularly *Streptococcus sanguis, Streptococcus oralis and Streptococcus mitis. Actinomyces species, Haemophili and Nisseria* species were also isolated during early plaque build up (28). It is conceivable that the subsequent development of plaque could be affected if the attachment and growth of these early colonizers were inhibited by antimicrobial agents (29). In an in-vitro study, nisin had been shown to be bactericidal to a wide range of organisms including *Actinomyces, Bacillus, Clostridium, Corynebacterium, Enterococcus, Gardnerella, Lactococcus, Listeria, Micrococcus, Mycobacterium, Propionihacterium, Streptococcus,* and *Staphylococcus* (21). The Probiotic mouthrinse may achieve its anti-plaque activity by inhibiting the growth and proliferation of these microorganisms on the tooth surface.

In this study, the reduction in plaque accumulation and gingivitis may also be due to a confounding effect known as the Hawthorne effect. The subjects participation in a clinical trial which involved prophylaxis and repeated dental examinations may, even if no active attempts were made to change the quality of the self-performed plaque control stimulated the subjects involved to improve their mechanical tooth cleaning measures. The subjects usually would improve their oral hygiene although they did not have the knowledge of the regimen administered to them. This was in agreement to another study by Brecx et.al (13) comparing the efficacy of Listerine, Meridol and Chlorhexidine on plaque and gingivitis, where it was demonstrated that mean PI scores in the placebo group decreased at day 7 due to the Hawthorne effect.

The efficacy of a mouthrinse is dependent on a few factors. One of the most important is its retention in the oral cavity. The overall retention of an antiplaque agent is determined by the strength and rate of association of the agent with its receptor sites and the accessibility of these sites. The substantivity of an antiplaque agent and its clearance from the oral cavity were determined by the rate of dissociation of the agent from the receptor sites and the salivary composition and flow rate (27). In this study, Probiotic antiplaque and antigingivitis activities may also be dependent on its retention and substantivity in the mouth, but this aspect of its properties was not investigated in this study and therefore need further exploration.

A novel approach in controlling unwanted microbial adhesion in clinical environments is to inhibit the initial attachment of bacteria, rather than trying to remove them once they have adhered. Previous investigations had established that antimicrobial peptides such as nisin can adsorb to surfaces and still retain sufficient activity to inhibit pathogenic bacteria (30, 31).

Bower et. al (31) evaluated the efficacy of adsorbed nisin films in preventing microbial attachment and biofilm formation. Nisin was adsorbed to silanized surfaces and these nisin-covered surfaces were exposed to a protein-free medium containing the pathogenic bacteria, *Listeria monocytogenes*. The study showed that nisin was bactericidal thus able to kill *Listeria monocytogenes.*

It was also proposed that Probiotic mouthrinse achieved its antiplaque activity by preventing the adhesion of bacteria to the tooth surface.

The success of adsorbed nisin as an antimicrobial agent for surfaces prompted further studies using medical devices such as endotracheal tubes and intravenous catheters. Endotracheal suction catheters that were exposed to nisin for as little as 10 seconds were able to retain a significant amount of antimicrobial activity (32). Longer adsorption time did not produce surfaces with substantially larger activities suggesting that contact time may not be critical when producing nisin-treated surfaces. When endotracheal tubes were dipped in nisin solutions and then challenged with three medically-relevant species of bacteria (*Staphylococcus aureus*, *S. epidermidis*, and *Streptococcus faecalis*), they were able to withstand bacterial colonization better than the untreated tubes which allowed attachment and growth of the pathogens (32). In the oral cavity, nisin can be clinically effective as an antiplaque agent as it can be adsorbed in a short time and it can be retained in the mouth after application to inhibit growth and proliferation of oral microorganisms.

A therapeutic mouthrinse usually contains an active ingredient that must be dissolved in the formulation. An antiplaque agent must be solublised in its delivery vehicle so that the rapid release into oral environment occurs particularly when the application time is short (27). The active ingredient must be soluble in an aqueous type of vehicle. Nisin has been shown to have a high aqueous solubility (33). In this Probiotic mouthrinse, nisin was highly solubilized in its aqueous delivery vehicle which can facilitate its release to the oral cavity during application as an antiplaque agent.

Guidelines have been established by the American Dental Association Council on Dental Therapeutics in 1986 for the evaluation of over-the-counter chemotherapeutic products for plaque and gingivitis control. ADA acceptance program requires that mouthrinses with plaque control claims must show concomitant gingivitis reduction as end point simply because plaque control is a therapeutic procedure basic to the prevention and treatment of periodontal disease. The ADA Council on Dental Therapeutics believes that because plaque is the etiologic agent for gingivitis and other oral diseases, the only accepted chemotherapeutic products that will be allowed to make plaque control or plaque modifications claims will be those that can also demonstrate a significant effect against gingivitis (34). In this study, Probiotic mouthrinse produced a significant gingivitis reduction of 25.2 % compared to placebo mouthrinse which produced only 5.8 % reduction. It showed that the Probiotic mouthrinse possessed an anti-gingivitis effect which can reduce gingival inflammation.

The pre-treatment debridement at day 0 and day 42 may influenced the findings of this study especially in the reduction of gingivitis seen in both the Probiotic and the placebo groups. This was in agreement with one of the studies conducted by Swedish researchers. Wennstrom and Lindhe (35) conducted a study in a group of periodontitis patients to assess the effect of the different types of mouthrinses (CHX, Sanguinarine and Placebo) who did and did not receive professional debridement (supragingival, subgingival scaling and root planing) during the treatment period.

CONCLUSIONS

In conclusion, this Probiotic mouthrinse is effective in reducing plaque accumulation and gingival inflammation in comparison to the placebo. Therefore, this Probiotic mouthrinse had potential therapeutic value and further longer-term term study of 6 months is recommended to determine its efficacy.

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