ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Salivarius K12 as A Probable Probiotic

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ABSTRACT

To evaluate the effect of the probiotic BLIS[R] K12 (Bacteriocin Like Inhibiting Substance) on the counts of *Streptococcus mutans* in the oral cavity. In this study 40 preschool children consisting of 16 girls and 24 boys were randomly selected. These children were divided blindly into a test and control group. The pre- salivary unstimulated saliva was collected in sterile salivary vials and subjected to microbiological assay to check for *S. mutans* counts for both groups. After introduction of probiotic in both groups post salivary samples were collected and again subjected to microbiological assay. A follow up collection of unstimulated saliva was done after 4 months again in both groups. A decreasing trend was seen in the *S. mutans* counts from pre to post salivary samples in the test group.The follow up results also showed a continual, statistically significant reduction in the counts in the test group. While in the control group there was no significant reduction. SK12 when used as a probiotic caused a significant reduction in the *S.mutans* count, follow up evaluation also showed a declining trend.
Keywords: Probiotics, saliva, *Streptococcus mutans*, *Streptococcus salivarius*

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ISSN: 0975-8585

INTRODUCTION

The initiation of caries and its progression requires the presence of host, substrate and bacteria concurrently to ferment the production of organic acids that leads to subsequent demineralization activity [1]. This process can be intercepted with the elimination of any one of these factors.

The various antibiotics and chemical agents used against cariogenic bacteria has proven clinical efficacy. But these are artificial agents and they kill a broad spectrum of microorganisms, some of which are beneficial to the host [1].

An alternative strategy to reduce cariogenic bacteria is bacteriotherapy/ replacement therapy [2] which involves the administering of live organisms in adequate amounts to confer a beneficial effect on the host. This concept was first explained by Ilya Metchnikoff (1908) [3]. Later, Lily and Stilwell (1965) coined the term Probiotics which is derived from Greek meaning "prolife" [4]. The probiotics used to confer health benefits on host are strains such as Lactobacillus reuteri, Weisella ciberia, Lactobacillus acidophilus and Lactobacillus fermentum.[4] However, since all of these organisms have the gastrointestinal tract as their inherent habitat, their induction into the oral environ proved to be transient and sustained long term benefits could not be reaped. Hence the use of these organisms in the oral cavity and therefore their efficacy in the oral context is quite questionable [3].

Another strain of microorganism that could be used as a probiotic is Salivarius K12 (SK12). SK12 is indigenous and a pioneer colonizer to the oral cavity and has the oral cavity as its inherent habitat. It is not an opportunistic pathogen notably. Hence, Streptococcus salivarius has been proposed as an excellent potential for use as a probiotic targeting the oral cavity [5]. SK12 has already been successfully employed in the treatment of throat infections, essentially against Streptococcus pyogenes, being available commercially as BLIS^{R} K12 (BLIS Technologies Ltd., Dunedin, New Zealand).

The present study was initiated on the hypothesis that when BLIS^{R} K12 used successfully as a throat guard against Streptococcus pyogenes probably could have an effect on the *S. mutans* in the oral cavity also.

MATERIALS AND METHODS

40 children consisting of 24 girls and 16 boys from a preschool nursery, aged between 3 and 4 years with good health and with no history of antibiotic therapy within three months were selected and grouped in to age and gender matched two groups of test and control. A history of antibiotic therapy would have altered the environment of the micro flora in the oral cavity[1]. This study was conducted at the Department of Pedodontics and Preventive Dentistry, Coorg Institute of Dental Sciences, Coorg, Karnataka, India with ethical committee clearances and informed written consent from parents of the subjects. Even though the study warranted follow up, all the subjects did not turn up for the 4 month follow up. Hence the data of 12 subjects of 8 girls and 4 boys were included in the test as well as control group and subjected

for further analyses. The probiotic used in this study was BLIS^{R} K12 throat guard which consists of 1 x 10^{9} CFU of *S. salivarius* K12 in the form of lozenges. The regimen included a 10 ml rinse of chlorhexidine gluconate after which at an interval of 2 hours, a single lozenge is administered for 4 times in a day. This regimen was repeated again on the second and third day. The control group was administered with probiotic curd containing lactobacillus acidophilus every four hours for three days.

The pre salivary samples of unstimulated saliva were collected in 5ml, sterile, salivary collection vials from the 12 selected children before the commencement of the probiotic regime by the method suggested by Colin Dawes [6]. On the following day, the entire regimen was followed in the test group and the control group. The unstimulated post salivary samples were collected at the end of the 3rd day in both groups and frozen.

The pre and post salivary samples were then subjected to microbiological assay. Mitis Salivarius agar was used as selective medium for the isolation and cultivation of Streptococcal spp. bacteria. The pre and post salivary samples were plated and cultured in a controlled environment for a period of 72 hours before the counts of *Streptococcus mutans* were taken.

All the pre and post salivary samples were tested for antibiotic sensitivity by standard diffusion method for Azithromycin, Rifampicin, Linozolid, Daptamycin, Teicoplanin, Ciprofloxacin, Vancomycin, Gentamycin, Penicillin and Oxacillin. No resistance was reported to the above mentioned drugs by the isolations.

All the forty children were then recalled after 4 months for follow up collection of salivary samples, but only twelve children in test group turned up or were without any antibiotic treatment. Twelve children from control group was also selected for further analyses. The data were subjected to statistical analysis using Student's t test comparing between test and control for each observation. Analysis of Variance (One Way ANOVA) comparing pre, post and 4 month follow up colony counts was also performed. Duncan's Multiple Range (DMR) Test was also employed as post hoc analysis to elucidate multiple comparisons. For all statistical evaluations, a two-tailed probability of value, < 0.05 was considered significant.

RESULTS AND DISCUSSION

RESULTS

Bacterial load was estimated using serial dilution method and n $\times 10^{3}$ was found suitable for the present *S. mutans* culture. The salivary samples from all the subjects were cultured and counted separately for pre; post and four month follow up treatment. The results are provided in Table 1.

A mean culture count of 218 $\times 10^{3}$ CFU were obtained for pretreatment, which showed a very high fluctuation with 36 to more than 1000 $\times 10^{3}$ CFU. After treatment the mean count came down to 25.83 $\times 10^{3}$ CFU without much deviation. Analysis of variance (One Way ANOVA)

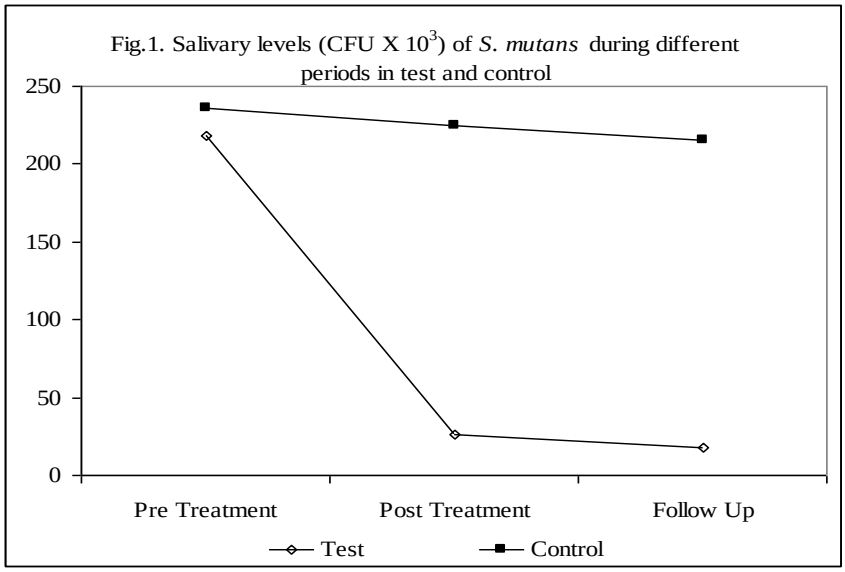
ISSN: 0975-8585

and post hoc comparison showed a highly significant (P<0.01) difference between pre and post treatment bacterial colony counts.

In the control group a mean culture count of 236.27 x 10^3CFU was obtained for pre-treatment. After treatment the mean count came down to 224.98 x 10^3 which was not highly significant (P< 0.05).

The salivary samples were subjected for bacterial load evaluation after 4 months and results showed that the *S. mutans* colony counts still reduced than post treatment assessment to 18 ×10^3 CFU, which was again significantly different from pretreatment but no significant difference was registered from post treatment bacterial load in the test group. While in the control group there was a slight drop in the counts from the post treatment but there was no significant difference.

The difference in the pre and post and follow up values of *S. mutans* in salivary samples showed a sustained declining trend with steep decrease from pre to post and still a low at the follow up after a period of 4 months (Fig 1).



DISCUSSION

Bacteriotherapy in the form of a probiotic with an inhibitory effect on oral pathogens is a promising concept, especially during childhood[1]. To serve as an effector strain in bacteriotherapy the microorganism must be non-virulent itself and should be able to compete successfully with the pathogenic microorganism either via competitive action and/or antibiotic action. *S. mutans* effector strains have been identified and show strong anti-*S mutans* activity. A disadvantage with the use of *S. mutans* effector strains is the cariogenic potential of these strains whereas *S. salivarius* is an alternative Streptococcus species which circumvents this detriment [5]. Many strains of lactobacillus have also being identified to hamper growth of oral

streptococci. But, unfortunately all these strains have shown only temporary reduction in S.mutans thus indicating the necessity of continual administration of the probiotic in order to achieve an effect. All these factors prompted the need for a probiotic against dental caries with a longer sustain

The prerequisites for the microorganism to be employed as a probiotic are whether the bacteria would exhibit antibiotic resistance; its metabolic activities could have the potential adverse effect on the host, furthermore could it exert inhibitory activity against other commensal microorganisms and whether they have the potential to carry particular virulence determinants. Hereof, a recent study has documented the safety data relating to SK12 and suggests that SK12 has excellent potential for use as a probiotic targeting the oral cavity [5].

In order to facilitate colonization of SK12, a preliminary step is to pretreat the individual by administering an antimicrobial agent such as Lactoperoxidase, green tea, pineapple juice(freeze dried) , or even follow a prescribed course of antibiotics such as penicillin, erythromycin or amoxicillin with the view to reduce the population levels of existing oral microbiota. Thereafter the intent of colonizing SK12 is achieved by administering SK12 to the depopulated environment of the oral cavity and essentially achieving the desired goal of replacement of the resident dental caries causing MS strains, particularly *S. mutans* in the individual [5].

A currently preferred protocol for bacteriotherapy with Salivarius K12 (SK12) comprises of pre-treatment by brushing teeth with chlorhexidine gel for 2 to 5 days (preferably 3 days), followed by administering a lozenge of SK12 1-4 hours (preferably 2 hours) after using the gel. This is then followed by the administration of further 2-5 lozenges, (preferably 3 lozenges through the day) at intervals of 1-4 hours, preferably every 2 hours. This protocol is continued for 2-4 days to facilitate colonization. Thereafter for maintenance purposes 1, 2, or 3 lozenges usually 1 to 2 lozenges are taken each day following ordinary tooth brushing. This regime could be continued for as long as is required [5].

However in our study BLIS^{R} K12 was administered according to the manufacturer's instructions wherein a chlorhexidine rinse is recommended at the start of the regimen followed by the administration of four lozenges per day at an interval of two hours for eight hours for the duration of three days as this pilot study was based on the hypothesis that BLIS K12 when used as throat guards against Streptococcus pyogenes probably also had an effect on the *S. mutans* counts in the oral cavity [5].

CONCLUSION

From this pilot study a significant reduction in *S. mutans* counts was noticed after administration of SK12. Nevertheless, in order to explain the concept of replacement therapy from this study, the identification of SK12 in the oral cavity after depopulating the oral cavity is to be established. However there is a definite, sustained and continual reduction in the *Streptococcus mutans* counts in the individuals after administering SK12 proving the fact that

there is an undeniable effect on *S. mutans* when SK12 is used as a probiotic. Irrefutable evidence to prove conclusively that SK12 is a potential probiotic for use in the oral cavity would entail a study design with a large sample size with long term follow up using the recommended dose and regimen of SK12.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the efforts of Mr. Vishnu (Microbiologist, MIMS, Calicut, Kerala), Mr. Shiby Mathew John (New Zealand) and the preschool children (Gonikoppal, South Coorg)

REFERENCES

[1]. Anderson M.H. Pediatr Dent 2006; 28(2):151-153.
[2]. Twetman Svante, Stecksen – Blicks Christina. Int J Pediatr Dent 2008; 18: 3-10.
[3]. Meurmann JH, Stamatova I. Probiotics: contributions to oral health. Oral Dis 2007 13(5); 443-451.
[4]. Asif Pasha, Asma, Dr Arun J. Chemical Index of Medical Science 1991.
[5]. Burton P Jeremy, Wescombe A Philip, Moore J Chris, Chilcott N Chris, John R Tagg. Appl Environ Microbiol 2006; 72(4): 3050-3053.
[6]. Dawes C. Factors influencing salivary flow rate and composition, Saliva and oral health. 2nd edition 1996; 27.
[7]. Sudhir R, Praveen P, Anantharaj A, Karthik V. Nigerian Med J 2012; 53(3): 135-139.