DOI: 10.1111/j.1365-263X.2007.00866.x

A probiotic lozenge administered medical device and its effect on salivary mutans streptococci and lactobacilli

ESBER ÇAGLAR, OZGUR ONDER KUSCU, SULE KAVALOGLU CILDIR, SENEM SELUI KUVVETLI & NUKET SANDALLI

Department of Paediatric Dentistry, Dental School, Yeditepe University, Istanbul, Turkey

International Journal of Paediatric Dentistry 2008; 18: 35–39

Background. Previous studies have suggested that lactobacilli-derived probiotics in dairy products may affect oral ecology, but the effects of different delivery methods have received little attention.

Aim. The aim of the present study was to investigate the effect of the probiotic *Lactobacillus reuteri*, delivered by a new medical device, on the levels of salivary mutans streptococci and lactobacilli in young women with high *Streptococcus mutans* counts.

Design. This is a randomized, double-blind, placebo-controlled study involving 20 healthy young women (aged 20 years): 10 as subjects and 10 as controls. The study subjects (Group A) sucked the medical device containing the probiotic lozenge with *L. reuteri* ATCC 55730/*L. reuteri* ATCC PTA 5289 (1.1×10^8 CFU) once daily for 10 days, while the control subjects (Group B) received placebo medical devices without bacteria. Salivary mutans streptococci and lactobacilli were enumerated with chair-side kits at baseline and 1 day after the final ingestion.

Results. Salivary *S. mutans* levels in the probiotic test group were significantly reduced, with statistical significance of reduction ($P < 0.05$).

Conclusions. A short-term daily ingestion of lactobacilli-derived probiotics delivered via medical device containing probiotic lozenge reduced the levels of salivary mutans.

Introduction

Probiotics are food supplements that contain live bacteria, which benefit people's digestive tract by maintaining a balanced gut flora[1–4]. The beneficial role of *Lactobacillus reuteri* ATCC 55730 in general health, immunomodulation, and disease protection has been observed and described in a number of studies[5–7]. Within dentistry, there are only a few studies investigating the effect of *L. reuteri* in the oral cavity. However, the effect of *L. reuteri* on one of the caries pathogens has been evaluated *in vitro* by Nikawa *et al.*[8], and consumption of yogurt containing *L. reuteri* SD 2112 (ATCC 55730) resulted in a significant growth inhibition of *Streptococcus mutans*, which was in contrast with other probiotic lactobacilli strains. In the studies mentioned above, dairy products such as milk, yogurt, and cheese have been selected as delivery vehicles for the selected bacteria. Probiotics can also be delivered by lozenges, powder, gelatine, straw, or tablets[1]; however, the best vehicle for probiotic delivery has yet to be identified[9].

Caglar *et al.*[10] investigated the effect of the probiotic bacterium *L. reuteri* ATCC 55730 on the levels of salivary mutans streptococci and lactobacilli in young adults when ingested via two different non-dairy delivery systems (straws and tablets). A significant reduction of the mutans streptococci levels was recorded after ingestion of the probiotic bacteria via both straw and tablets, which was in contrast to the placebo controls. Recently, our research group evaluated the effect of probiotic chewing gums containing two strains of *L. reuteri* (ATCC 55730 and ATCC PTA 5289) on levels of salivary mutans streptococci and lactobacilli in young adults[11]. Daily chewing of the gum containing probiotic bacteria reduced the levels of salivary mutans streptococci significantly.

In daily routines, the administration of probiotics to breastfed children and toddlers is difficult. The pacifier can be used as a vehicle to transport essential nutrients to the infant's

Correspondence to:
Dr Esber Caglar, Department of Pediatric Dentistry, School of Dentistry, Yeditepe University, Bagdat cad 238, Goztepe 34728 Istanbul, Turkey. Tel. +90 216 3636044/323; Fax: +90 216 3636211; E-mail: caglares@yahoo.com

body, but the pacifier is also a risk for mother–child *S. mutans* transmission. Clinical data show that the later the primary teeth are colonized by mutans streptococci, the less likely that dental caries will develop, if at all. Since up to 90% of teething children develop a sucking habit[12], pacifiers were recently designed to release preventive agents into the oral cavity[13–16]. There is also some evidence that pacifiers may do less harm to the dentition, particularly because pacifier habits are often spontaneously shed at about 2–4 years of age[12,15,17]. For this purpose, a slow-release medical device with a pouch has been developed. To our knowledge, the possible effect of probiotics on the oral microflora after sucking a medical device has not been reported. The aim of the present study was to investigate the effect of the probiotic bacterium *L. reuteri* on the levels of salivary mutans streptococci and lactobacilli in young women who used the new slow-release medical device containing *L. reuteri*. The null hypothesis was that the dissolution of probiotics from a lozenge administered medical device would not alter salivary mutans streptococci and lactobacilli.

Materials and methods

The study was a randomized, double-blind, placebo-controlled design over a time course of 10 days, with 15 min of intervention per day. The volunteer group consisted of 20 healthy young women (aged 20 years) in order to present a sample size of potential mothers with a high caries risk. Ten of these volunteers acted as study subjects (Group A), and 10 as controls (Group B). For ethical reasons and assuming that mothers would prefer their babies not to participate in such a study, our pilot design vehicle was tested on adult volunteers of young child-bearing age. Only volunteers with high counts of salivary mutans streptococci ($\geq 10^5$ CFU) were included in the study before randomization. The study protocol was in accordance with the Helsinki Declaration of Human Rights and was approved by the Ethical Committee at the School of Dentistry, Yeditepe University, Istanbul, Turkey. The subjects received both oral and written information about the study and signed their informed consent. All participants included were healthy and had not used antibiotics or probiotics within the 2-week washout period prior to the study.

Fig. 1. Probiotic lozenge administered medical device.

The present medical device contains a pouch in which the probiotic or placebo lozenge can be inserted in (Fig. 1). It has the standard 10 holes, each 0.5 mm in diameter, where probiotic lozenge totally dissolves into the oral cavity in approximately 10–12 min (investigated clinically and microbiologically in a pilot study). The medical devices (the authors are currently seeking patent from Turkish Patent Institute) were specially designed and manufactured by Wee (Burda Ticaret, Istanbul, Turkey) and met the requirements of the European Union and Turk Standartlari Enstitusu (TSE) standards[16,18]. The medical devices were differentiated by colour (blue: probiotic; and pink: placebo). The administered probiotic lozenge (Biogaia AB, Stockholm, Sweden) has the following composition: freeze-dried *L. reuteri* ATCC 55730 : ATCC PTA 5289 10 : 1, and isomalt. The placebo lozenges were identical in size, form, and taste, but without live bacteria. The daily sucking was performed for 15 min at noon, and no tooth brushing was allowed for at least 1 h after sucking.

Samplings of paraffin-stimulated whole saliva were carried out immediately before and after the 10-day trial. After the participants thoroughly rinsed their mouths with water, the saliva was collected (during a course of

5 min) directly into a graded test tube. The counts of salivary mutans streptococci and lactobacilli were evaluated using CRT (Ivoclar Vivadent AG, Schaan, Liechtenstein) as described earlier[19,20].

The data were processed statistically with the GraphPad Prisma V.3 program. Chi-squared test was applied for intergroup comparison and a *P*-value of less than 0.05 was considered statistically significant. Post- and and pre-treatment values within each regimen were compared with McNemar test and a *P*-value of less than 0.05 was considered statistically significant.

Results

The pre- and post-treatment levels of salivary mutans streptococci and lactobacilli are shown in Tables 1 and 2. All subjects had high counts of salivary mutans streptococci at baseline while most of them had high counts of salivary lactobacilli. During the test period with the probiotic test medical device, seven subjects exhibited decreased scores for salivary mutans streptococci, while three subjects had unchanged scores. The corresponding values for the control regimen were one and six subjects, while one subject displayed a one-step increase. Salivary *S. mutans* levels in the probiotic test group were significantly reduced, with statistical significance of reduction ($P < 0.05$). The probiotic and placebo groups also behaved differently as salivary *S. mutans* in probiotic test group was found to be reduced ($P < 0.05$) following the 10-day sucking of the test medical device in contrast to the control medial device.

Regarding salivary lactobacilli, no similar trend towards reduced bacterial counts was seen after the 10-day period of probiotic medical device ($P > 0.05$).

Table 1. Distribution of salivary mutans streptococci at baseline and after 10 days of sucking the medical device (*N* = 20).

		Probiotic group	Placebo group	*P*
Baseline (pre-treatment)				
mutans streptococci score, CFU/mL				
0	No growth	0	0	
1	$< 10^5$	0	0	NS
2	10^5–10^6	6	6	
3	$> 10^6$	4	4	
10 days (post-treatment)				
0	No growth	0	0	
1	$< 10^5$	3	0	0.016*
2	10^5–10^6	7	5	
3	$> 10^6$	0	5	
P		0.016**	NS	

*χ^2-test. **McNemar test.

Table 2. Distribution of salivary lactobacilli at baseline and after 10 days of sucking the medical device (*N* = 20).

		Probiotic group	Placebo group	*P*
Baseline (pre-treatment)				
lactobacilli, CFU/mL				
0	$\leq 10^3$	0	0	
1	10^4	5	5	NS
2	10^5	5	3	
3	$\geq 10^6$	0	2	
10 days (post-treatment)				
0	$\leq 10^3$	0	0	
1	10^4	4	2	NS
2	10^5	5	5	
3	$\geq 10^6$	1	3	
P		NS	NS	

χ^2 and McNemar tests.

Discussion

It seems easier to affect or change levels of caries-associated bacteria at the time of its colonization compared to later in life when the flora is firmly established. Novel control strategies could control the microbial activities using biofilm to prevent colonization of selected organisms while supporting growth of other selected ones. The major route of early acquisition and colonization of mutans streptococci in humans is a vertical transmission from mother to child. Recently, Lindquist and Emilson[21] showed in their longitudinal clinical study that new genotypes and a gain–loss pattern were noted especially in the children, but also in their mothers regarding mutans streptococci. In fact, probiotics in the infant oral cavity can compete with newly acquired caries-related micro-organisms. Our research group has recently established a colonization study. Twenty-five volunteers consumed a chewable tablet that contained *L. reuteri* ATCC 55730 (10^8 CFU/tablet) during a 14-day trial period. The number of *L. reuteri* carriers decreased gradually, and after 1 week only 8% of the subjects harboured the bacterium. After 5 weeks *L. reuteri* was not detected in any of

the subjects. Some strains most likely have oral effects without colonizing the oral cavity. In the present study, a multistrain probiotic (*L. reuteri* ATCC 55730/*L. reuteri* ATCC PTA 5289) was used. While a monostrain has to overcome barriers presented by the host and its endogenous microflora, multistrain probiotics have a greater divergency, and the enhanced chance of survival of at least one strain[22].

However, one should note that colonization in infancy is rather complicated compared to the one in adult oral ecological system. In the present study, because of ethical reasons, the target was potential mothers with high caries risk, as pacifiers are known transmission vehicles between mother and infant. The present study is unique as the study population is limited to high caries risk group of a specific age band, the time of the study was short as fresh probiotic applications would not reflect daily life, and probiotic lozenge administered medical devices have not been used previously in the literature. In conclusion, the dissolution of probiotics from a lozenge administered medical device has been shown to significantly reduce salivary mutans streptococci.

What this paper adds

- Dairy foods such as milk, yogurt, and cheese have usually been selected as vehicles for probiotics; however, pacifiers in terms of medical devices can also be used as vehicles.
- A short-term daily ingestion of lactobacilli-derived probiotics delivered by medical device containing probiotic lozenge reduced the levels of salivary *S. mutans*.

Why this paper is important to paediatric dentists

- In daily routines, the administration of probiotics to breastfed children and toddlers is difficult. The pacifier can be used as a vehicle to transport essential nutrients to the infant's body.

Acknowledgements

We would like to thank our volunteers for their participation, Professor Dr Richard Welbury (Glasgow, Scotland), Associate Professor Dr Fulya Isik (Istanbul, Turkey) and Ms Idil Aktan (Philadelphia, PA, USA) for revising the English text, Ms Rana Konyalıoglu (Istanbul, Turkey) for her statistical expertise, Ms Cigdem Kumarki (Ivoclar-Vivadent-Guney Dis Deposu, Istanbul, Turkey) and Mr Altay Uludamar (Ivoclar-Vivadent Marketing, Istanbul, Turkey) for supplying chair-side saliva tests, Mr Eamonn Connolly (Biogaia, Stockholm, Sweden) for providing probiotic lozenges, and Mr Akin Guney (Wee, Burda Ticaret, Istanbul, Turkey) for manufacturing, testing, and supplying the novel medical devices.

References

1 Çaglar E, Kargul B, Tanboga I. Bacteriotherapy and probiotics' role on oral health. *Oral Dis* 2005; **11**: 131–137.
2 Çaglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with Bifidobacterium DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. *Acta Odontol Scand* 2005; **63**: 317–320.
3 Lenoir-Wijnkoop I, Sanders ME, Cabana MD *et al*. Probiotic and prebiotic influence beyond the intestinal tract. *Nutr Rev* 2007, in press.
4 Çaglar E, Sandalli N, Twetman S. Probiotische Produkte und orale Gesundheit. *Prophylaxe Impuls* 2006; **10**: 62–68.
5 Valeur N, Engel P, Carbajal N, Connolly E, Ladefoged K. Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. *Appl Environ Microbiol* 2004; **70**: 1176–1181.
6 Casas IA, Dobrogosz WJ. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. *Microbial Ecol Health Dis* 2000; **12**: 247–285.
7 Weizman Z, Asli G, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. *Pediatrics* 2005; **115**: 5–9.
8 Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K. *Lactobacillus reuteri* in bovine milk fermented decreases the oral carriage of mutans streptococci. *Int J Food Microbiol* 2004; **95**: 219–223.
9 Meurman JH. Probiotics: do they have a role in oral medicine and dentistry? *Eur J Oral Sci* 2005; **113**: 188–196.
10 Çaglar E, Kavaloglu S, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium *Lactobacillus reuteri* ATCC 55730 by straws or tablets. *Acta Odont Scand* 2006; **64**: 314–318.
11 Çaglar E, Kavaloglu SC, Kuscu OO, Sandalli N, Holgerson PL, Twetman S. Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutans streptococci and lactobacilli. *Clin Oral Invest* 2007, Epub ahead of print, doi: 10.1007/s00784-007-0129-9.
12 Çaglar E, Larsson E, Andersson EM, Hauge M, Ogaard B, Bishara S. Feeding, artificial sucking habits and malocclusions in 3 years old girls in different regions of the world. *J Dent Child* 2005; **72**: 25–30.
13 Suhonen J, Sener B, Bucher W, Lutz F. Release of preventive agents from pacifiers in vitro. An

introduction to a novel preventive measure. *Schweiz Monatsschr Zahnmed* 1994; **104**: 946–951.
14 Aaltonen AS, Suhonen JT, Tenovuo J, Inkila-Saari I. Efficacy of a slow-release device containing fluoride, xylitol and sorbitol in preventing infant caries. *Acta Odontol Scand* 2000; **58**: 285–292.
15 Larsson E, Bishara S. *The Influence of Oral Habits on the Developing Dentition and Their Treatment*, 2nd edn. Skaraborg Institute, Skaraborg, Sweden, 2003.
16 European Standard. 1400-1: 2002. Child use and care articles – soothers for babies and young children. Part 1: general safety requirements and product information. Official Journal of the European Union, C100, 24 April 2004. Brussels: EU, 2004: 20.
17 Kargul B, Caglar E, Tanboga I. Feeding practices and sucking habits in Istanbul children: a clinical study of prevalence and effects on dentition. *J OHDMBSC* 2003; **1**: 20–25.
18 Turk Standartları Enstitüsü. TS 4270-2 EN 1400-2: 2003. Çocuk Kullaným ve Bakým Esyalarý-Bebekler ve Küçük Çocuklar Için Emzikler Bölüm 2: Mekanik Özellikler ve Deneyler. Turkish version of EU guidelines Europen Standard. 1400-1: 2002.
19 Bratthall D. Dental caries: intervened–interrupted–interpreted. Concluding remarks and cariography. *Eur J Oral Sci* 1996; **104**: 486–491.
20 Gwinnett AJ, Ceen RF. Plaque distribution on bonded brackets: a scanning microscope study. *Am J Orthod* 1979; **75**: 667–677.
21 Lindquist B, Emilson CG. Colonization of *Streptococcus mutans* and *Streptococcus sobrinus* genotypes and caries development in children to mothers harboring both species. *Caries Res* 2004; **38**: 95–103.
22 Timmerman HM. *Multispecies probiotics: composition and functionality*. PhD Thesis, Universitet Utrecht, Utrecht, the Netherlands, 2006.