European Review for Medical and Pharmacological Sciences 2016; 20: 4601-4606

Effect of administration of *Streptococcus salivarius* K12 on the occurrence of streptococcal pharyngo-tonsillitis, scarlet fever and acute otitis media in 3 years old children

F. DI PIERRO[1], M. COLOMBO[2], M.G. GIULIANI[3], M.L. DANZA[4], I. BASILE[4], T. BOLLANI[5], A.M. CONTI[6], A. ZANVIT[7], A.S. ROTTOLI[8]

[1]Scientific Department, Velleja Research, Milan, Italy
[2]ATS, District 5, Milan, Italy
[3]ATS District 8, Milan, Italy
[4]ATS, District 6, Milan, Italy
[5]ATS, District 4, Milan, Italy
[6]ATS, District 7, Milan, Italy
[7]Biological Dentistry Department, Italian Stomatology Institute, Milan, Italy
[8]Pediatric Department, "Uboldo Hospital", Cernusco S/N, Milan, Italy

Abstract. – OBJECTIVE: ***Streptococcus salivarius*** **K12 (BLIS K12) is a probiotic strain strongly antagonistic to the growth of *Streptococcus pyogenes*, the most important bacterial cause of pharyngeal infections in humans. Shown to colonize the oral cavity and to be safe for human use, BLIS K12 has previously been reported to reduce pharyngo-tonsillitis episodes in children or adults known to have experienced recurrent streptococcal infection. The present study was focussed upon evaluating the role of BLIS K12 in the control of streptococcal disease and acute otitis media in children attending the first year of kindergarten.**

PATIENTS AND METHODS: By randomization, 222 enrolled children attending the first year of kindergarten were divided into a treated group (N = 111) receiving for 6 months a daily treatment with BLIS K12 (Bactoblis®) and a control group (N = 111) who were monitored as untreated controls. During the 6 months of treatment and 3 months of follow-up, the children were evaluated for treatment tolerance, and for episodes of streptococcal pharyngo-tonsillitis, scarlet fever and acute otitis media.

RESULTS: During the 6-month trial (N = 111 per group) the incidence of streptococcal pharyngo-tonsillitis, scarlet fever and acute otitis media was approximately 16%, 9% and 44% respectively in the treated group and 48%, 4% and 80% in the control group. During the 3-months follow-up (N = 29 per group) the corresponding rates of infection were 15%, 0% and 12% in the treated group and 26%, 6% and 36% in the controls. No apparent side effects were detected in the treated group either during treatment or follow-up. All of the enrolled children completed the study.

CONCLUSIONS: The daily administration of BLIS K12 to children attending their first year of kindergarten was associated with a significant reduction in episodes of streptococcal pharyngitis and acute otitis media. No protection against scarlet fever was detected.

Key Words:
Paediatric infections, Pharyngo-tonsillitis, Scarlet fever, Acute otitis media, Blis K12, Bactoblis®.

Introduction

Streptococcus salivarius K12 (hereafter BLIS K12) is a probiotic strain shown to strongly inhibit the *in vitro* growth of *Streptococcus pyogenes*, *Streptococcus pneumonia, Haemophilus influenza, and Moraxella catarrhalis* principal etiological agents respectively of bacterial pharyngo-tonsillitis and acute otitis media[1,2]. This antagonism seems to be due to the release of the lantibiotics salivaricin A2 and salivaricin B[3]. After oral administration, BLIS K12 colonizes the oral cavity, nasopharynx and adenoids[4] persisting there for up to one month after the last dose[5]. It is antibiotic-sensitive[6] and has a thoroughly-investigated safety profile[7]. From a clinical perspective, administration of BLIS K12 has been shown to

Corresponding Author: Francesco Di Pierro, Ph.D; e-mail f.dipierro@vellejaresearch.com

reduce re-occurrences of streptococcal and viral pharyngo-tonsillitis as well as acute and secretory otitis media[8-12]. A common denominator of these previous studies has been the enrolment of subjects having a history of recurrent streptococcal, infection, with no less than three episodes per year as demonstrated by culture growth of *Streptococcus pyogenes*. By contrast, in the present study, we have evaluated whether BLIS K12 could provide protection for children who were attending their first year of kindergarten and who had no recent history of recurrent streptococcal pharyngo-tonsillitis or acute otitis media.

Patients and Methods

Product

BLIS K12 was formulated as slowly-dissolving oral tablets by SIIT (Trezzano S/N, Milan, Italy) and notified to the Italian Ministry of Health as Bactoblis® by Omeopiacenza (Pontenure, Italy), according to the provisions of law No 169 of 2004, on July 5th, 2011 (notification number: 53435). The preparation Bactoblis® used in the clinical trial contained no less than 1 billion CFU/tablet of *Streptococcus salivarius* K12 (Blis Technologies Ltd., New Zealand).

Clinical Trial

This multicentre, open-label, randomized, controlled clinical trial was conducted on 222 children (116 females and 106 males aged 33-45 months) enrolled in the area of Milan (Italy). The children were treated (N = 111) or untreated (N = 111), between September 2015 and March 2016 with Bactoblis®. Between April and July 2016 (N = 29 per group) a 90-days follow-up was performed. The trial was conducted according to the criteria set by the Declaration of Helsinki and with the approval of the local (Milan, Italy) Ethics Committee. The parents of all the participants in the study were informed of the trial methods and signed the appropriate consent and privacy policy documents.

Inclusion Criteria

At the time of enrolment, all of the children were around 3 years of age and were soon to attend the first year of kindergarten. All participants were free of streptococcal disease, as established by a rapid throat swab test for group A streptococcus. None were clinically ill on enrolment.

Exclusion Criteria

Children were excluded from the study if they were immunocompromised, had undergone tonsillectomy or had an indication for adeno-tonsillectomy. Other exclusion criteria included a history of rheumatic disorders, bronchospasm and/or a diagnosis of asthma and/or allergy; a diagnosed respiratory or significant systemic disorder. Also excluded were children who were either undergoing current pharmacological therapies to prevent recurrent respiratory infections or who presented with conditions that could favour the development of acute otitis media, including severe atopy, acquired or congenital immunodeficiency, cleft palate, a chronically ruptured eardrum, craniofacial abnormalities or obstructive adenoids, sleep apnoea syndrome or placement of tympanostomy tubes.

Study Pattern

All individuals enrolled were first subjected to a general medical examination and pharyngeal swab (Test Strep-A, Gima, Gessate, Italy) and then were randomized by tossed coin in two groups: a treated group daily administered, for 6 months, with BLIS K12 in the form of Bactoblis® tablets and an untreated group not receiving any treatment and simply monitored as control group. The parents of the children in the BLIS K12 group were instructed on how to use the product. The tablets were to be administered for 180 consecutive days. The children had to let one tablet dissolve slowly in the mouth immediately before going to sleep, after brushing their teeth. The children were to be carefully instructed not to chew the tablets or to swallow them whole. Furthermore, they should not drink or swallow anything else just following the use of the product. For the trial period, it was requested that at the first sign of any oropharyngeal symptoms of infection the children should be brought to the clinic for an immediate medical examination and pharyngeal swab test. In the case of a positive result, treatment was prescribed. The prescribed therapy for streptococcal infection was a combination of amoxicillin and clavulanic acid to be administered for 10 days. Following antibiotic therapy, treatment with BLIS K12 was resumed and continued until the scheduled 180th day of the study. Infections accompanied by pharyngo-laryngeal pain and/or a fever were treated with acetaminophen or ibuprofen. Diagnosis of scarlet fever[13] and acute otitis[14] media was done on the basis of the microbial and clinical evidence and

performed by trained investigators. Any other pathologies possibly occurring during the study were treated according to the recommendations of the Italian Paediatric guidelines.

Study Aims

The present study aimed to evaluate the following: (1) the onset of side effects or symptoms of toxicity while the product was being administered; (2) the efficacy of BLIS K12 in the prevention of *Streptococcus pyogenes* infections (pharyngo-tonsillitis and scarlet fever) during 6-months of treatment and a 3-month follow-up period; 3) the efficacy of BLIS K12 in reducing the occurrence of acute otitis media.

Statistical Analysis

The equivalence of the two subject groups was determined using Fisher's exact test and the two-tailed Wilcoxon-Mann-Whitney test respectively. The difference in terms of numbers of streptococcal pharyngo-tonsillitis, scarlet fever and acute otitis media episodes was determined using the two-tailed Wilcoxon-Mann-Whitney test. Statistical software used was JMP 10 for Mac OsX and the threshold for statistical significance was 95%.

Results

Children (N = 222) attending the first year of kindergarten in the area of Milan (Italy) were enrolled and randomized into two groups of 111 children, one of which was treated, and the other not treated for 6-months by daily administration of Bactoblis® tablets to effect slow release into the oral cavity of the anti-streptococcal probiotic strain BLIS K12. After this period some of the children (N = 29 per group) continued to be monitored for a further three months (follow-up). None of the enrolled children presented characteristics of streptococcal recurrence and/or were assessed as otitis media-prone. Compliance throughout the 180 days of Bactoblis® treatment was assessed as very good; no side effects were reported and none of the children were withdrawn from the study (data not shown). Since, as shown in Table I, the two groups did not exhibit significantly different characteristics in terms of age, sex, previous streptococcal or acute otitis media episodes, ethnicity, pneumococcal vaccine, type of delivery, weight at birth, type of feeding, presence of older brothers and previous attendance at nursery school, their backgrounds were considered to be comparable. Table II shows the number of children diagnosed with streptococcal pharyngo-tonsillitis, scarlet fever and acute otitis media during the 6-month treatment period. Eighteen of 111 (16.2%) of the treated group and 54 of 111 (48.6%) in the control group were diagnosed with streptococcal throat infections. On the other hand, no statistical difference was found for episodes of scarlet fever (10 of the treated group versus 7 of the controls).

Table I. Features* of the children (222) enrolled and ending the study.

	Treated	Untreated
Total number	111	111
Males	50	56
Age of males (months ± SD)	36 ± 3.2	35 ± 3.0
Females	61	55
Age of females (months ± SD)	34 ± 3.0	35 ± 3.6
Previous streptococcal episodes	16	14
Previous AOM episodes	4	3
Italians	75	69
Arabs/Africans	30	33
Asians	6	9
Pneumococcal vaccine^	9	10
Naturally delivered	72	75
Caesarean delivered	39	36
Weight at birth (kg ± SD)	3.2 ± 0.7	3.3 ± 0.8
Breastfed	39	42
With older brothers	51	56
Nursery attenders	45	51

*None of the features is significantly different between the two groups; ^PCV13; SD: standard deviation.

Table II. Number of children with pharyngo-tonsillitis (PT), scarlet fever (SF) and acute otitis media (AOM) during the 6-months treatment period in the two study groups (N=111/group).

	PT	%	SF	%	AOM	%
Treated	18*	16.2	10	9.0	49*	44.1
Control	54	48.6	7	6.3	89	80.2

*$p < 0.01$ *vs*. control.

A diagnosis of acute otitis media was made for 49 (44.1%) of the 111 Bactoblis®-treated children and this was significantly fewer than the 89 (80.2%) of 111 control group children having a positive diagnosis. Further analysis of the diagnosed episodes of streptococcal throat infection (Table III) shows that in the treated group 16 children experienced a single infection, one child had two infections and another child had three episodes during the 6-month treatment period, for a total of 21 episodes. By comparison, in the control group 67 streptococcal throat infections were diagnosed, with 45 children each having a single episode, five with two episodes and four children having three episodes. A similar situation was observed for total episodes of acute otitis media: in the treated group 46 children had a single episode, two children each experienced two episodes and another child had three episodes giving a total of 53 episodes versus 101 episodes in the control group, with 80 children having one episode, six having two episodes and three with three episodes reported. During the 3-month follow-up period (Table IV) five (17.2%) of the 29 children in the treated group experienced streptococcal pharyngo-tonsillitis and four (13.8%) were reported to have single episodes of acute otitis media. None of these children developed scarlet fever. No significant difference in the occurrence of streptococcal infections during the follow-up period was observed for the control group, with eight (27.6) of the 29 children experiencing infection and two cases of scarlet fever reported. Nevertheless, a significant difference was found for reported cases of acute otitis media with 12 (41.3%) of the 29 children infected in the control group.

Table III. Total episodes of pharyngo-tonsillitis (PT) and acute otitis media (AOM) during the 6-months treatment period in the two study groups (N=111/group). Into brackets number of children with 1, 2 or 3 episodes.

	PT (1, 2, 3 episodes)	AOM (1, 2, 3 episodes)
Treated	21* (16, 1, 1)	53* (46, 2, 1)
Control	67 (45, 5, 4)	101 (80, 6, 3)

*$p < 0.01$ *vs*. control.

Discussion

Achieving oral health benefits from probiotic therapy has recently become possible with the development of novel probiotics such as BLIS K12 selected from the oral cavity commensal species *Streptococcus salivarius*. The BLIS K12 strain was originally isolated from the oral cavity of a young child who had no recent experience of *S. pyogenes* infection. The lantibiotic bacteriocins produced by this strain have subsequently been shown to be inhibitory not only to *S. pyogenes* but also to other oral cavity bacterial pathogens associated with acute otitis media[2] and halitosis[15,16]. Previous investigations have demonstrated that the oral administration to healthy volunteers of BLIS K12 reduces IL-8 plasma con-

Table IV. Number of children with pharyngo-tonsillitis (PT), scarlet fever (SF) and acute otitis media (AOM) during the 3-months follow-up in the two study groups (N=29/group).

	PT	%	SF	%	AOM	%
Treated	5	17.2	0	0	4*	13.8
Control	8	27.6	2	6.9	12	41.3

*$p < 0.051$ *vs*. control.

centrations and increases salivary γ-interferon.[2] These modulations may also rationally account for the anti-inflammatory, immuno-modulating and anti-viral activity recently observed by our group[17,18]. All of the clinical trials performed to date on BLIS K12 have aimed to reduce streptococcal pharyngo-tonsillitis or acute otitis media episodes in subjects already having a clear history of recurrent streptococcal disease or otitis media. It has not however yet been established whether the prophylactic administration of BLIS K12 can help provide a clinical benefit to individuals not known to have a predilection to streptococcal infection or otitis media. The results of the current study provide some preliminary support for this proposition. BLIS K12 prophylaxis given to 3 years old children attending the first year of kindergarten and who did not yet seem to be either streptococcal pharyngitis or acute otitis media prone, appeared to highlight a reduction in episodes of both of these infections, the treated children being protected by about 60% and 50% respectively by comparison with the children in the control group.

Also, the consideration of subjects who experienced infection recurrences appears to show a beneficial effect from the use of BLIS K12. Indeed, only two subjects of the treated group versus nine of the controls were found to have recurrent streptococcal throat infections. Also, only three of the treated group had recurrences of acute otitis media by comparison with nine of the control subjects. Perhaps anomalously the BLIS K12 treatment did not seem to create any benefit in terms of prevention of scarlet fever episodes. Although this pathology occurs as a result of *Streptococcus pyogenes* infection, its main characteristic, skin rash, is due to the release of erythrogenic toxins[19] that the bacterium produces when is infected by a specific phage[20]. One possible hypothesis is that *Streptococcus salivarius* K12 more effectively antagonizes the replication of *Streptococcus pyogenes* strains that are not phage-infected. Another possible theory is that although strain K12 may have killed the phage-infected streptococci, the latter may still have released rash-inducing concentrations of toxins into the throat tissues. The prophylactic role against streptococcal throat infections and episodes of otitis media played by BLIS K12 during the 6-month treatment period seems also to have been maintained for acute otitis media during the 3-month washout period. Even in this case, there is a possible bias, since the relatively small number of subjects (29 per group), who accepted to be monitored during the follow-up could have led to an absence of significant results in terms of episodes of streptococcal throat infections.

Conclusions

Limitations of the present study include the absence of blind randomisation and a totally untreated control group. Nevertheless, the results appear to demonstrate for the first time that the prophylactic administration of BLIS K12 could generate positive clinical outcomes even in very young children who have not been pre-selected as recurrent streptococcal infection or acute otitis media-prone. Follow-up studies should be conducted to establish whether BLIS K12 is for some reason specifically non-protective against development of scarlet fever. These studies should include *in vitro* tests of the action of BLIS K12 against scarlet fever toxin-positive and -negative strains of *Streptococcus pyogenes* and also a double-blind, placebo-controlled trial containing a large number of young scarlet fever vulnerable subjects.

Conflict of Interest

FDP is the main formulator of the tested product and he is involved in the Scientific Council of the Company (Omeopiacenza®) trading the tested product. The other authors do not report any conflict of interest.

References

1) Wescombe PA, Burton JP, Cadieux PA, Klesse NA, Hyink O, Heng NC, Chilcott CN, Reid G, Tagg JR. Megaplasmids encode differing combinations of lantibiotics in Streptococcus salivarius. Antonie Van Leeuwenhoek 2006; 90: 269-280.

2) Wescombe PA, Hale JD, Heng NC, Tagg JR. Developing oral probiotics from Streptococcus salivarius. Future Microbiol 2012; 7: 1355-1371.

3) Hyink O, Wescombe PA, Upton M, Ragland N, Burton JP, Tagg. Salivaricin A2 and the novel lantibiotic salivaricin B are encoded at adjacent loci on a 190-kilobase transmissible megaplasmid in the oral probiotic strain Streptococcus salivarius K12. Appl Environ Microbiol 2007; 73: 1107-1113.

4) Power DA, Burton JP, Chilcott CN, Dawes PJ, Tagg JR. Preliminary investigations of the colonisation of upper respiratory tract tissues of infants using

a paediatric formulation of the oral probiotic Streptococcus salivarius K12. Eur J Clin Microbiol Infect Dis 2008; 27: 1261-1263.

5) Horz HP, Meinelt A, Houben B, Conrads G. Distribution and persistence of probiotic Streptococcus salivarius K12 in the human oral cavity as determined by real-time quantitative polymerase chain reaction. Oral Microbiol Immunol 2007; 22: 126-130.
6) Burton JP, Wescombe PA, Moore CJ, Chilcott CN, Tagg JR. Safety assessment of the oral cavity probiotic Streptococcus salivarius K12. Appl Environ Microbiol 2006; 72: 3050-3053.
7) Burton JP, Cowley S, Simon RR, McKinney J, Wescombe PA, Tagg JR. Evaluation of safety and human tolerance of the oral probiotic Streptococcus salivarius K12: a randomized, placebo-controlled, double-blind study. Food Chem Toxicol 2011; 49: 2356-2364.
8) Di Pierro F, Adami T, Rapacioli G, Giardini N, Streitberger C. Clinical evaluation of the oral probiotic Streptococcus salivarius K12 in the prevention of recurrent pharyngitis and/or tonsillitis caused by Streptococcus pyogenes in adults. Expert Opin Biol Ther 2013; 13: 339-343.
9) Di Pierro F, Donato G, Fomia F, Adami T, Careddu D, Cassandro C, Albera R. Preliminary pediatric clinical evaluation of the oral probiotic Streptococcus salivarius K12 in preventing recurrent pharyngitis and/or tonsillitis caused by Streptococcus pyogenes and recurrent acute otitis media. Int J Gen Med 2012; 5: 991-997.
10) Gregori G, Righi O, Risso P, Boiardi G, Demuru G, Ferzetti A, Galli A, Ghisoni M, Lenzini S, Marenghi C, Mura C, Sacchetti R, Suzzani L. Reduction of group A beta-hemolytic streptococcus pharyngotonsillar infections associated with use of the oral probiotic Streptococcus salivarius K12: a retrospective observational study. Ther Clin Risk Manag 2016; 12: 87-92.
11) Di Pierro F, Di Pasquale D, Di Cicco M. Oral use of Streptococcus salivarius K12 in children with secretory otitis media: preliminary results of a pilot, uncontrolled study. Int J Gen Med 2015; 8: 303-308.
12) Di Pierro F, Colombo M, Zanvit A, Risso P, Rottoli AS. Use of Streptococcus salivarius K12 in the prevention of streptococcal and viral pharyngotonsillitis in children. Drug Healthc Patient Saf 2014; 6: 15-20.
13) Wessels MR. Pharyngitis and Scarlet Fever. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. Streptococcus pyogenes: Basic Biology to Clinical Manifestations. Oklahoma City (OK): University of Oklahoma Health Sciences Center, 2016.
14) Lieberthal AS, Carroll AE, Chonmaitree T, Ganiats TG, Hoberman A, Jackson MA, Joffe MD, Miller DT, Rosenfeld RM, Sevilla XD. The diagnosis and management of acute otitis media. Pediatrics 2013; 131: e964-999.
15) Burton JP, Chilcott CN, Moore CJ, Speiser G, Tagg JR. A preliminary study of the effect of probiotic Streptococcus salivarius K12 on oral malodour parameters. J Appl Microbiol 2006; 100: 754-764.
16) Masdea L, Kulik EM, Hauser-Gerspach I, Ramseier AM, Filippi A, Waltimo T. Antimicrobial activity of Streptococcus salivarius K12 on bacteria involved in oral malodour. Arch Oral Biol 2012; 57: 1041-1047.
17) Di Pierro F, Campana A, Panatta ML, Antenucci V, De Vincentiis G. Possible use of Streptococcus salivarius K12 in counteracting PFAPA Syndrome. Ped Intern. Alter Integr Med 2016. Submitted.
18) Di Pierro F, Colombo M, Zanvit A, Rottoli AS. Positive clinical outcomes derived from using Streptococcus salivarius K12 to prevent streptococcal pharyngo-tonsillitis in children. Drug, Healthcare Pat Saf. Submitted July 2016.
19) Ralph AP, Carapetis JR. Group A streptococcal diseases and their global burden. Curr Top Microbiol Immunol 2013; 368: 1-27.
20) Smith NL, Taylor EJ, Lindsay AM, Charnock SJ, Turkenburg JP, Dodson EJ, Davies GJ, Black GW. Structure of a group A streptococcal phage-encoded virulence factor reveals a catalytically active triple-stranded beta-helix. Proc Natl Acad Sci U S A 2005; 102: 17652-17657.