Clinical reduction of S. mutans in pre-school children using a novel liquorice root extract lollipop: a pilot study

M.C. Peters, J.A. Tallman***, T.M. Braun**, J.J. Jacobson****
**School of Dentistry, **School of Public Health, University of Michigan, Ann Arbor, ***Dental Research and Data Institute, Delta Dental of MI, OH, IN, Okemos, Michigan, USA*

Abstract

AIM: To determine the clinical effect of a simple herbal caries-prevention protocol aimed at reduction of Streptococcus mutans (SM) in young children in a pre-school setting. **STUDY DESIGN:** Proof-of-principle pilot study. **METHODS:** To prove the concept this pilot study delivered a clinical intervention using sugar-free lollipops containing liquorice root extract. Regimen: Supervised herbal lollipops, twice daily for 3 weeks. Species-specific monoclonal antibody testing of saliva provided SM counts. Children were grouped in high, medium and low caries-risk using baseline SM-levels as risk-indicator. Bacterial numbers at baseline, during intervention, and for 9 weeks post-intervention were compared. **STATISTICS:** SM levels were analysed using GEE modelling. **RESULTS:** High-risk children showed the steepest early decrease in mean log-SM (P<. 001). At end of a follow-up period, the log-SM decrease moved the high-risk group down to moderate-risk level. High-risk children showed a decrease in fitted mean SM% not seen in other groups (P<. 001). The decrease reached a nadir around 22-days post-intervention. Twice-daily use of herbal lollipop significantly reduced both number and relative percent of SM in high-risk children. SM numbers were reduced for 22 days after the last lollipop, stabilized and then began to rebound. **CONCLUSION:** A potential for simple effective caries-prevention for high-risk children has been demonstrated. Encouraging results warrant randomised clinical trials (RCT) of liquorice root in herbal lollipops or alternative modes of delivery.

Introduction

Dental caries is one of the most common diseases of childhood; 5 times as common as asthma and 7 times as hay fever [NIDCR National Institute of Dental and Craniofacial Research, 2000]. Recently, the Centers for Disease Control (USA) alarmingly reported that the prevalence of caries in young children had increased. [CDC Centers for Disease Control, 2009]. 'Healthy People 2010' is a comprehensive framework for improving the health of Americans, built on the foundation of several decades of prior initiatives. It has two overarching goals, to 'increase the quality and years of healthy life' and 'eliminate health disparities'. The 'Healthy People 2010' target of reducing the number of children with dental caries was slipping out of control as the proportion of young children who ever had dental caries in their primary teeth increased from 18% (1988-1994) to 24% (1999-2004), moving away from the 'Healthy People 2010' target of 11% [ODPHP Office of Disease Prevention and Health Promotion, 2000].

Nearly 60% of children in the USA age 5 to 17 years-old have a decayed or filled primary and/or decayed, filled or missing permanent tooth [NIDCR National Institute of Dental and Craniofacial Research, 2000]. Furthermore, individuals living below the national poverty level experience more decay than those above the poverty line, despite significant overall progress in reducing dental caries. For example, in the State of Michigan, one-third of Head Start children aged 3-5 years-old have developed dental caries [MDCH Michigan Department of Community Health, 2005]. Launched in 1965 as part of Lyndon Johnson's 'War on Poverty', the federally funded Head Start is a national USA program that promotes children being ready for school by enhancing the social, emotional and cognitive development of children. This is through the provision of educational, health, nutritional, social and other services to enrolled children and families. It is one of the longest-running programs to address systemic poverty in the USA [ACF Administration for Children and Families, 2010]. As of late 2005, more than 22 million pre-school aged children have participated in Head Start. Identifying and developing simple and effective prevention protocols for young children is greatly needed.

Mutans streptococci (SM) are a principal factor in the initiation of dental caries and contribute to the progression of the disease [Fitzgerald and Keyes, 1960]. There is good evidence of a strong correlation between the proportions of SM in plaque or saliva and current or future caries experience [Loesche et al., 1975]. Therefore, the proportion of SM of plaque or saliva counts may serve as an indicator for caries activity state and caries risk or susceptibility.

A promising intervention of the caries process is the use of herbs, specifically liquorice, for the reduction of SM. A sugar-free, orange flavoured lollipop was developed (C3 Jian/ Intelliherb Inc., Inglewood, CA), containing an extract of liquorice root that has been shown to target and kill SM in-vitro [He et al., 2006] The lollipops contain hydrogenated starch hydrolysate, citric acid, natural and artificial flavouring, food colouring, Glycyrrhiza uralensis (GU) and artificial sweetener. GU, commonly referred to as liquorice root, contains active

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Postal address: Dr. Tilly Peters, Department of Cariology, Restorative Sciences and Endodontics. School of Dentistry University of Michigan, 1100 N University, Ann Arbor, MI 48109, USA.
Email: mcpete@umich.edu:

antimicrobial compounds. With minimum inhibitory concentrations of 1 and 2 μg/mL Glycyrrhizol A and B, extracted from the roots of GU, exhibited potent antibacterial activity against SM [He et al., 2006]. Liquorice root has been used worldwide as a sweetener in food and medicine production for many years and is listed in the USA by the Food and Drug Administration (FDA) as Generally Recognized as Safe (GRAS).

The unique approach of the delivery system – an orange flavoured, sugar free herbal lollipop – (Kavidy KopsTM, Dr. John's Candies, Grand Rapids, MI, USA) raises the hope of a simple, effective way to deliver a targeted intervention to young children who are at risk for dental caries. This is the first report of a pilot clinical trial of the liquorice root lollipop in children enrolled in a Head Start program.

The null hypothesis was that Head Start children after a twice-daily oral care regime of herbal lollipops would show no difference in pathogen SM counts compared with baseline. To prove the concept, this pilot clinical trial aimed to determine if a twice daily oral regime of herbal lollipops for three weeks: (1) lowered the numbers of SM from a baseline measurement, (2) if so, did the SM rebound and over what period of time, and, (3) if this oral health prevention regime could be conducted in a Head Start classroom setting.

Materials and Methods

Subjects. As parents of the children wanted their child to receive the herbal lollipop and not potentially a placebo, the study was designed to prove the concept and each child served as their own internal control for this pilot clinical trial. [Schmidt, 2006] The research protocol and all printed materials, including translations in Spanish and Hmong, were reviewed and approved through this board. Following staff training, written informed consent by parent or guardian was obtained for each participating child during the initial home visit before the school year started or when the child was brought to their classroom. A colour pamphlet using pictures of local Head Start children was developed and provided to each family to explain the project. All children in the participating Head Start classrooms were eligible and had the choice to participate or not. Although individual oral health status was not recorded in this pilot study, a significant dental caries prevalence rate of 38% has been reported among children enrolled in Head Start programs [Siegal et al., 2004]. Children with allergies to liquorice, flavouring or colouring agents were excluded.

The children enrolled were in six full-day classrooms, 2-5 years of age (2% was 2 years old; 37% 3 yrs, 57% 4 yrs; 4% 5 yrs), 44% F and 56% M (40% Caucasian, 28% African American, 17% Hispanic, 12% Multi Racial, 2% Asian, and 1% Native American). Translations of the materials and translators were available as needed.

SM assays. Because selective culture assays usually underestimate the actual number of SM, can be inaccurate and have high false-positive or false-negative data, a species-specific monoclonal antibody test of saliva was used to measure total numbers of bacteria and of SM [Gu et al., 2002] and have been recognized as an excellent diagnostic tool. Researchers at Oral Microbiology Laboratory (University of California, Los Angeles) recently developed highly species-specific monoclonal antibodies against SM. In conjunction with fluorescent microscopy and flow cytometric techniques, antibodies allow quick, low-cost assays that detect salivary SM with nearly 100% sensitivity and specificity and which count the number of bacteria with great accuracy [Gu F et al., 2002].

Saliva samples. These were collected early morning as soon as children arrived at school and before they eat breakfast. The children were asked to spit normal (unstimulated) saliva in a disposable cup, Fig.1. Using a pipette 0.5 ml of saliva was transferred into a tube with fixing solution, and mixed for 5 seconds by shaking. The first, unstimulated baseline saliva measure was obtained the week prior to starting the lollipops regimen (day 0). Subsequent unstimulated saliva collections were taken prior to lollipops use on days 7, 9, 11, 14, 18, 21, and 25 the last day of lollipops. Follow-up saliva samples (days 28-84) were collected every Monday for 9 additional weeks.

Figure 1. Photograph showing child providing saliva sample.

Lollipop use. Each child was given a lollipop, (Fig 2a) containing 15 mg liquorice root, every morning and afternoon of each school day for 3 weeks. There were no eating or drinking restrictions before or after using the lollipops. Children were seated at tables or in a story circle to help ensure the lollipops were consumed safely, (Fig.2b). Educational materials and suggested activities were provided to each classroom to help the children understand that the lollipops were not the same as others and were not sweets/candy. Puzzles, storybooks, videos, puppets, and games kept the children interested in the study and compliant with the protocol.

Classroom teaching staff supervised the children and recorded which ones successfully dissolved their lollipop, defined as holding the lollipop in their mouth for 10 minutes. A study coordinator supervised unstimulated saliva sample

collection according to the testing kits protocol (UCLA Oral Microbiology Laboratory, OML). The samples were labeled with unique identifiers to protect and maintain privacy and anonymity during the laboratory testing and data evaluation. Each week samples were mailed to OML in padded envelopes. Refrigeration was not required for this testing product, making storage and shipping much simpler.

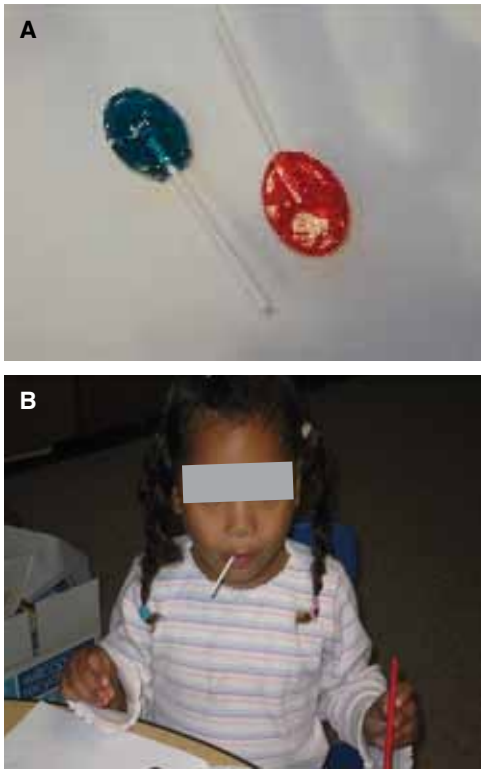

Figure 2. Photographs showing: A. liquorice containing fruit flavoured lollipops; B. test subject using lollipop during school lesson.

Statistical Methods. Without any useful clinical data regarding the effect of this novel intervention, a planned sample size of 100 children at enrollment was estimated, rather than calculated to power a specific hypothesis [Schmidt, 2006]. Nonetheless, the data of the 66 children whose data could be analysed, supplied sufficient information allowing an examination of the effects of the oral care intervention with sufficient accuracy and supplying evidence in support of the concept, useful for the design of a larger randomised, controlled study.

The study was designed to collect pathogen levels at 17 distinct time points for each child. However, pathogen levels were missing for some children, either due to inability to provide an adequate saliva sample for evaluation, missing some days or dropping out of the study entirely. The missing data were not imputed and were assumed to be missing completely at random. The observed baseline pathogen levels taken a week prior to starting the lollipops were used as risk-indicator, placing each child into one of three risk categories: high, medium, and low. Based on the distribution of salivary SM counts in nearly 2,000 saliva samples collected from children (age 2 to 16), [Gu et al., 2002] samples were divided into three levels: high, medium, and low SM counts. Children with <100,000 (1×10^5) SM cells/ml of saliva were considered low caries risk; 100,000 to 500,000 (1×10^5 to 5×10^5) SM cells/ml as medium risk, and individuals with >500,000 (5×10^5) cells/ml were high risk. Baseline pathogen levels were missing for 21 children, who were excluded from the analyses comparing the three risk groups.

Observed pathogen levels were modelled (numbers of total bacteria and of SM) for each child with generalized estimating equations (GEE) assuming independence among the multiple pathogen levels measured for each child (i.e. an independence 'working' correlation matrix) using software package R, [R Development Core Team, 2008]. GEE with an independence working correlation matrix allowed us to correctly estimate the average pathogen levels over time using standard linear regression methods while also correcting the statistical significance of our results (i.e. using 'robust' standard errors) to account for the (unknown) temporal correlation of pathogen levels within-child [Liang and Zeger, 1986]. GEE was used to model both mean log-transformed SM (log-SM) and mean SM% (ratio SM/total bacteria) over time for all three-risk groups. The two GEE models contained main effects for risk group, linear and quadratic effects of time, and the interaction of all time effects with risk group, creating an average curve over time for each of the three risk groups. Overall models were also fitted, pooling all 66 children with full data sets together into a single group. Significance of time and group effects were assessed using Wald tests, with statistical significance defined as a P-value less than 0.05.

Results

Subjects. There were 100 children consented and entered into the trial. A classroom of 13 children only received lollipops in the morning, precluding them from being included with those following the 2/day research protocol. Furthermore, baseline pathogen levels were not measured on 21 children due to insufficient amounts of saliva, further reducing the sample size to 66 children. Using baseline pathogen levels (day 0) to indicate future caries risk, there were 12 low-risk, 37 moderate-risk and 17 high-risk children. No adverse events or side effects were reported and the children liked the flavour of the lollipops. Compliance in the classroom setting was assured, although at the start of the study collection of sufficient saliva presented some challenges.

Log-SM. The top plot in Figure 3 shows the actual longitudinal pattern of mean log-SM for the three risk groups; the shaded area delineates the three distinct periods of the study (0-7 = baseline), (7-28 = lollipop intervention), and (29-84 = follow up period). The important finding in Figure 3 was that the high-risk children had the steepest early decrease in mean log-SM (P<. 001). Due to variability both within and among children, no distinct trend was apparent, although an

overall decrease in mean log-SM over the study period can be seen. The bottom plot in Figure 3 shows the fitted longitudinal patterns of mean log-SM for all children, regardless of baseline risk as well as stratified by baseline risk group. Furthermore, this decrease in high-risk children was large enough, so that by the end of follow-up, the average log-SM for high-risk children was at a level defined as moderate-risk. Although the top plot in Figure 3 also displays an increase in mean log-SM for low-risk children, this curve was not statistically significantly different from a flat line.

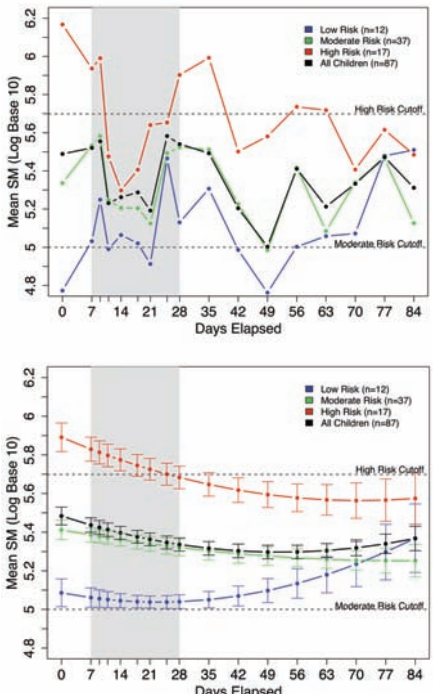

Figure 3. Actual (top) and fitted (bottom) longitudinal plots of average (log base 10) SM numbers (±1 SEM) for all children and stratified by risk group. The shaded area delineates the 3 weeks of liquorice extract lollipop intervention.

The top plot in Figure 4 shows the actual longitudinal pattern in mean SM% for each risk group, and the bottom plot in Figure 4 shows the fitted longitudinal pattern in mean SM% for each risk group over time. Similar to Figure 3, there was a decrease seen for the high-risk children in the fitted means that did not occur in moderate and low-risk children (P<. 001). However, the decrease in fitted mean SM% reached a nadir around day 50 (22 days after the last lollipop), at which point the fitted mean numbers of SM began to increase, indicating that a longer period or continued administration of the herbal lollipop would be needed to obtain a durable, long-term benefit.

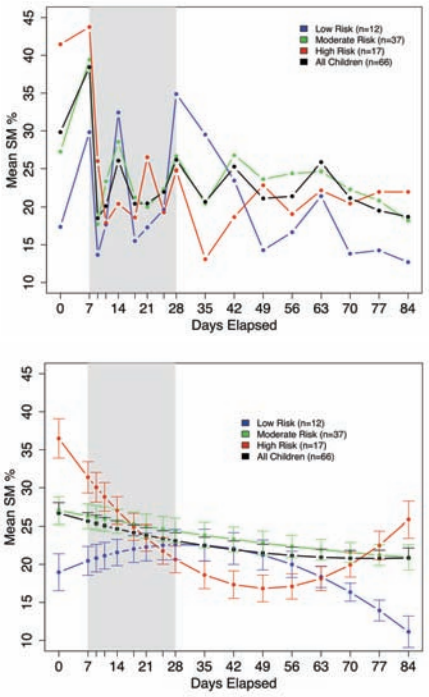

Figure 4. Actual (top) and fitted (bottom) longitudinal plots of average SM % (±1 SEM) for all children and stratified by risk group. The shaded area delineates the 3 weeks of a liquorice extract lollipop intervention.

Discussion

This is the first clinical report of the use of GU, the active ingredient in the liquorice root extract, in a simple, effective prevention protocol to reduce the numbers of SM. The pilot clinical trial was carried out in a caries-prone pre-school population to prove the concept. A unique delivery system, a lollipop, was used to administer the active ingredient.

Limitations. Before results are discussed, several limitations of this pilot clinical trial must be presented. Firstly, as noted previously, a true control group in which a placebo was used was not possible. All of the parents/legal guardians wanted their child to receive the herbal lollipop instead of a placebo. Consequently, each child served as their own internal control with a baseline measurement (day 0) taken during the week prior to starting the lollipops. Evaluating and interpreting the outcome of this study, inevitably two common confounding conditions need to be taken into account. The first is the 'Hawthorn Effect', in which study participants behave differently during the study period simply in response to the fact that they are being studied [McCarney et al., 2007]. In our case, the observed effect (decrease in log-SM) may be due to this change in behaviour just by being part of a clinical study, rather than the liquorice extract. Examples might include oral hygiene practices changing, or a change in diet during the study period. Secondly, regression toward the mean, could also affect the results. In general, over time there is a normal regression of high counts and low counts to move

to the centre. Thus this regression to the mean could be confounding the observed results. The collection of unstimulated saliva, however, proved to be problematic for some of the young children resulting in insufficient amounts of saliva for testing. This unexpected effect might be resolved in future studies by using stimulated saliva.

Liquorice effect. Given the above limitations, the results suggest that use of a twice-a-day regimen of liquorice extract lollipops for three weeks significantly reduced the number (Fig. 3) and relative percent of SM (Fig. 4) in a high-risk child population. There were no documented adverse events or side effects reported. When given the lollipops, the children were compliant and liked their flavour. This pilot study provides evidence about the feasibility of conducting such a study in a low socio-economic Head Start classroom setting.

High-risk groups. A combination of history of caries, dietary habits and SM gave the best prediction of caries in a study of Finnish toddlers [Pienihakkinen et al., 2004]. While the accuracy of the measurements was low, [Thenisch et al., 2006] enumeration of caries-associated bacteria (accuracy of 75%) was the best single predictor (sensitivity 0.69, specificity 0.78) in the toddlers [Pienihakkinen et al., 2004]. Although caries experience is the strongest predictor as children increase in age, [Grindefjord M et al., 1995] one would like to predict the caries-risk before the disease manifests itself. The current study distributed children into three groups according to their SM counts as they are associated with a considerable increase in caries-risk [Thenisch et al., 2006] and thus may be considered a risk-indicator, indirectly associated with the disease [Twetman and Fontana, 2009].

Significance. Due to the small group size of low risk children (12) estimation of the significance of the increase in SM levels in this group is difficult. But the effect on moderate and low risk children appeared to be minimal to non-existent (Figs 3 and 4). Further, the impact of reducing the numbers of SM in high-risk children appeared to be effective for about 22 days after lollipop use, at which point, the drop in log-SM stabilized and began to rebound (Fig. 4). The decrease in log-SM in the high-risk population was large enough to move the high-risk children to the moderate-risk level (Fig. 3). A future prospective trial measuring subsequent reduction in caries activity might provide the definitive answer to the clinical relevance of this short-term reduction in SM. The potential effect of increased duration of the intervention, or repeated administration after rebounce will be part of future studies.

The results of this pilot clinical trial are encouraging and a randomised controlled clinical trial (RCT) is warranted to confirm these early findings [Schmidt, 2006]. This small-scale study showed preliminary evidence of efficacy of the herbal lollipop, measured by a surrogate endpoint, i.e. reduction in SM, as a proxy measure or biomarker for anti-caries efficacy.

In addition to SM counts and comprehensive clinical criteria, also measuring the pH of the plaque on the surface of the teeth would assist in understanding the effect of GU on the dental caries process. Future RCT studies should include SM counts, plaque pH measurements and detailed clinical criteria including non-cavitated lesion assessment.

Conclusions

Twice-daily use of herbal liquorice lollipop significantly reduced, for 22 days, the number and relative percent of S. mutans in high-risk children which stabilized and began to rebound. The potential for a simple, effective caries-prevention protocol for high-risk children is demonstrated in this pilot trial.

Acknowledgments

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