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Rebalancing the Caries Microbiome Dysbiosis: Targeted Treatment and Sugar Alcohols

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Abstract:

Dental caries is a disease that results from microbiome dysbiosis with the involvement of multiple cariogenic species, including mutans streptococci (MS), lactobacilli, *Scardovia wiggsiae* and several *Actinomyces* species that have the cariogenic traits of acid production and acid tolerance. Sugar consumption also plays an important role interacting with microbiome dysbiosis determining the fate of caries development. In addition, the MS transmission that encompasses multiple sources, can have long-term impacts on the oral microbiome and caries development in children. Intervention in MS transmission in early childhood may promote effective long-term caries prevention. Anti-caries regimens aimed against the above mechanisms will be important for successful caries management. Xylitol and erythritol may serve as good components of anti-caries regimens as oral microbiome modifiers, sugar substitutes, and agents to prevent MS transmission in early childhood with both oral and systemic benefits. Further studies are needed to elucidate the mechanism of the anti-caries effects of xylitol and erythritol with consideration of their impacts on the microbiome and bacterial virulence, in addition to cariogenic bacteria levels as well as their benefits for overall health. On the other hand, the anti-caries agent, C16G2, specifically targeting *S. mutans,* the most common cariogenic bacterial species, has shown good safety for short-term oral topical use and promising effects in reducing *S. mutans in vitro* and *in vivo* with the promotion of oral commensal bacteria. Future study on its anti-caries effect will need to include its long-term impact on the oral microbiome and effects on other important cariogenic bacteria.

Keywords: Microbial ecology, Caries detection/diagnosis/prevention, Caries treatment, Bacterial virulence, Infectious disease(s)

Introduction

Dental caries is a multifactorial infectious disease that is characterized by microbiome dysbiosis with the elevation of cariogenic bacteria (Loesche 1986; Tanner et al. 2016). In the oral cavity, tooth surfaces are always covered by dental plaque. In the presence of sucrose and other fermentable carbohydrates, dynamic mineral loss (demineralization) is initiated by acid produced by oral bacteria metabolizing fermentable carbohydrates. The demineralized tooth structure can be repaired by remineralization when acid production diminishes as carbohydrate substrates are exhausted or acids are neutralized by intrinsic or extrinsic buffering agents in dental plaque. Balancing the equilibrium between demineralization and remineralization is the key for initiation, progression, or reversal of dental caries (Takahashi and Nyvad 2011; Young and Featherstone 2013).

Although multiple biological risk factors contribute to the caries balance, microbiome dysbiosis interacting with diet plays a critical role in the fate of dental caries development (Takahashi and Nyvad 2011; Tanner et al. 2016; Young and Featherstone 2013). Restorative dental treatment does not alter the cariogenic bacteria loading (microbiome dysbiosis) in the rest of the mouth and is often followed by continuing caries development in high caries-risk populations (Chaffee et al. 2016; Featherstone et al. 2012; Hughes et al. 2012; Tanner et al. 2011a; Zhan et al. 2006). Therefore, anti-caries treatments aimed to rebalance the dysbiosis of the oral microbiome are needed for successful caries management and prevention. This paper focuses on microbiome dysbiosis related to dental caries, discusses factors that influence the microbiome dysbiosis that leads to caries development, as well as anti-caries approaches by either targeted antimicrobial treatment against *S. mutans*, one of the main cariogenic bacteria, or polyols, such as xylitol and erythritol that are aimed at correcting oral microbiome dysbiosis.

Microbiome dysbiosis in dental caries

The mutans streptococci (MS) group is the most studied and well-accepted cariogenic bacteria collection of species. Both culture-based and DNA/RNA based studies have confirmed MS as one of the main cariogenic bacterial groups with *S.*

mutans and *S. sobrinus* as the two main subspecies in humans (Hughes et al. 2012; Loesche 1986; Tanner 2015; Tanner et al. 2011a; Tanner et al. 2016; Tanner et al. 2011b; Tanzer et al. 2001):

1) elevated oral MS levels have been detected in subjects prior to dental caries development (Leverett et al. 1993);

2) high MS levels have been observed in dental plaque or stimulated saliva in children or adults with initial or active decay (Loesche 1986; Tanner et al. 2011b; Tanner et al. 2012);

3) clinical trials have shown that reduction or elimination of MS resulted in caries prevention and relapse of MS dysbiosis resulted in new caries development (Featherstone et al. 2012; Laitala et al. 2013; Tanzer et al. 2001; Zhan et al. 2006);

4) animal studies indicated that MS inoculation induced the most amount of dental caries in both smooth surfaces and pits and fissures (Loesche 1986).

Therefore, there has been considerable attention given seeking treatment regimens to reduce or eliminate MS for caries prevention.

However, recent DNA/RNA based studies have expanded the microbiology in dental caries to include not only more cariogenic bacterial species but also the commensal bacteria associated with healthy subjects. Tanner et al recently presented a comprehensive review on the caries microbiome summarizing projects conducted in the Forsyth Institute in Boston, United States. Their studies indicated that dental caries is a result of dysbiosis of acid-producing and acid-tolerant bacteria with a close relationship to a frequent sugary or carbohydrate diet. In addition to the strong correlation of MS with dental caries (Hughes et al. 2012; Tanner et al. 2012), they also indicated a wide diversity of the caries microbiome, including the association of *Actinomyces* and related species with caries (Tanner 2015), especially a new species *Scardovia wiggsiae* in the *Actinomyces/Bifidobacterium* family and several other *Actinomyces* species (Hughes et al. 2012; Kressirer et al. 2017). Therefore, anti-caries treatment may be more logical in focusing on correcting the microbiome dysbiosis than on eliminating a single pathological species. Further studies are also needed to confirm the roles of other cariogenic bacteria in caries initiation, progression and management and to identify effective chair-side tools to monitor caries microbiome dysbiosis and to guide clinicians to effectively manage dental caries chemically.

Targeted Antimicrobial Treatment against *Streptococcus Mutans*

A pheromone-guided "smart" antimicrobial peptide, namely a specifically targeted antimicrobial peptide C16G2, has been developed for caries prevention. C16G2 is targeted at killing *S. mutans,* the most prevalent MS species in humans, based on the concept that this will lead to rebalancing the caries microbiome dysbiosis for an effective anti-caries effect. C16G2 consists of a targeting domain that is a truncated *S. mutans* competence stimulating peptide (CSP_{C16}), which bonds effectively on *S. mutans* cells, and a killing domain, G2, a truncated broad-spectrum killing peptide (novispirin G10). The two domains are linked by a flexible tri-glycine linker region (Eckert et al. 2012). C16G2 targetedly kills *S. mutans* without a significant effect on other commensal bacteria *in vitro* (Eckert et al. 2012; Guo et al. 2015). Use of C16G2 rinse for 4 days significantly reduced *S. mutans* in plaque and saliva, accompanied by an increase of *S. sanguinis* and *S. gordonii* levels, resting pH as well as reduced acid formation in dental plaque *in vivo* (Eckert et al. 2012). The completed Phase I and II studies showed good safety data for C16G2 in the oral cavity and confirmed that *S. mutans* levels were rapidly reduced, and remained low, even after cessation of therapy (Eckert et al. in press). These results show the potential of the effectiveness of targeted treatment on caries prevention and its benefits on rebalancing the oral microbiome through targeting the main cariogenic bacteria.

One concern is that C16G2 only targets *S. mutans* while caries microbiome dysbiosis involves multiple cariogenic species. For example, *S. sobrinus*, the other main species in the MS group, is commonly found in high-risk individuals (Kohler et al. 1995; Loesche 1986; Tanzer et al. 2001). Studies suggest that *S. sobrinus* may be more cariogenic than *S. mutans*. *S. sobrinus* strains are usually more acidogenic, produce structurally different and more abundant polysaccharide, and adhere better to smooth tooth surfaces (Kohler et al. 1995). The presence of *S. sobrinus* might be a stronger indicator of future caries risk and might place the patients at higher risk than *S. mutans* alone. In addition, newly detected *Scardovia wiggsiae* and several *Actinomyces* species have been shown to play important roles in caries formation when MS is absent (Hughes et al. 2012; Kressirer et al. 2017). In these cases, a targeted treatment only against *S. mutans* may not be effective. Secondly, *in vitro* and pilot studies have shown effective elimination of *S. mutans* from the oral biofilm by C16G2. It may also be important to consider that *S. mutans* is a part of the normal indigenous oral flora. Therefore, further monitoring on potential microbiome ecology consequences after elimination of *S. mutans* from oral flora is needed. The focus of anti-caries treatment may be best achieved by re-balancing the dysbiosis rather than elimination of *S. mutans* or any other single species. These concerns will need to be investigated in the Phase III clinical studies in humans.

Sugar Intake and the Microbiome Dysbiosis

Frequent carbohydrate ingestion plays a significant role in modifying the oral microbiome. Takahashi and Nyvad proposed an ecological hypothesis for caries microbiome dysbiosis with three reversible stages (Figure 1):

1) a dynamic stable stage on sound enamel where plaque contains mainly non-mutans streptococci and Actinomyces with mild and infrequent acidification and demineralization/remineralization shifting towards net mineral gain;

2) an acidogenic stage when increasing sugar/fermentable carbohydrate frequency results in aciduric and acidogenic adaptation of the non-mutans bacteria, a selective shift of aciduric bacteria, and a shift of demineralization/remineralization balance toward net mineral loss and initiation/progression of dental caries;

3) an aciduric stage, in which prolonged acidic conditions result in predominant colonization of aciduric and acidogenic bacteria including mutans streptococci, lactobacilli, as well as aciduric non-mutans streptococci, Actinomyces, bifidobacteria, and yeasts, and prolonged net mineral loss and progression of dental caries (Takahashi and Nyvad 2011).

This hypothesis is supported by the observations that, on sound enamel surfaces or in caries-free subjects, there are generally low levels of MS, lactobacilli, and other aciduric non-mutans streptococci in dental plaque with a less acidogenic and aciduric environment (Khoo et al. 2005; Loesche 1986; Tanzer et al. 2001). The aciduric and acidogenic bacteria, including MS and non-mutans species, increase in early enamel white spot lesions or before clinical lesions were detected (Leverett et al. 1993; Simon-Soro et al. 2013; Takahashi and Nyvad 2011; Tanner et al. 2012). Lastly, in subjects with active caries, MS and non-mutans aciduric and acigodgenic bacteria become dominant, accompanied by decreased microbiome diversity (Loesche 1986; Tanner et al. 2011b; Tanner et al. 2012). Frequent fermentable carbohydrate and sucrose intake plays a leading role in the shift of caries microbiome dysbiosis to more aciduric flora with an increase of MS and *lactobacilli* and decrease of *S. sanguis* while restricted sugar in the diet lowered the salivary *lactobacilli* and plaque MS (Loesche 1986; Simon-Soro et al. 2013; Takahashi and Nyvad 2011; Tanzer et al. 2001).

In addition to acid formation, sucrose also influences the microbiome by providing the main substrate for glucosyltransferases (GTFs) to synthesize extracellular polysaccharide (EPS). EPS formed *in situ* provides a matrix to enmesh the microorganisms in three- dimensional multicellular structures that are firmly attached to teeth. The EPS enriched biofilm limits diffusion, prolongs acidification processes in the presence of sugar, and creates a barrier to protect bacteria from antimicrobial treatment (Loesche 1986; Simon-Soro et al. 2013). Given the significant roles of sugar in caries formation and caries microbiome dysbiosis, sugar substitutes should be a significant part of anti-caries therapy in high-risk subjects.

Transmission of MS and its relationship to microbiome dysbiosis

Timing for transmission and colonization of MS also affects microbiome dysbiosis. For high-risk young children, the critical time for MS transmission and colonization is from birth to three years (Li and Caufield 1995; Wan et al. 2003). Early colonization of *S. sanguis* was associated with delayed MS colonization and MS colonization can be accompanied by decreased levels of commensal bacteria such as *S. sanguinis (Caufield et al. 2000; Simon-Soro et al. 2013)*. Further, children with early colonization of MS at or before age 4, not only had high levels of MS but were also more likely to develop new caries than those who did not colonize or colonized later

(Alaluusua and Renkonen 1983). Therefore, early intervention to delay MS transmission can be a strategy for long-term caries prevention in children. Studies on maternal use of xylitol have shown strong evidence for delaying and reducing MS colonization as well as long-term caries prevention in children (Soderling 2009).

However, although transmission of MS from mother to child is widely accepted as a main source of early MS acquisition in children (Li and Caufield 1995), the maternal transmission rate of MS ranges from 33 to 100% with other sources of MS transmission being documented within and beyond the family (Domejean et al. 2010; Li and Caufield 1995; Lindquist and Emilson 2004; Mitchell et al. 2009; Zhan et al. 2012c). MS transmission from father to child and between spouses are well documented (Emanuelsson 2001). Our group studied multi-generational MS transmission in young children with early childhood caries in maternal-grandmother-mother-child triads. Ten maternal-grandmother-mother-child triads were recruited with maternal grandmothers as the primary care-givers for the children. Saliva samples were collected from each member of the triads and MS were cultured on Mitis Salivarius Sucrose Bacitracin agar and five MS colonies were isolated for AP-PCR assay with Primers OPA 05 and OPA 13 for genotyping. The results demonstrated all possible MS transmission combinations (Figure 2) in the triads with the grandmother-child transmission rate as the highest as 55% (Table 1). Other studies have also demonstrated horizontal MS transmission in nursery schools and elementary school from playmates who had close-contact with the child (Domejean et al. 2010). Recently, two studies demonstrated that ~90% of MS genotypes in 2-5 year old children with early childhood caries were from non-maternal sources even when mothers were the primary caregivers (Mitchell et al. 2009; Zhan et al. 2012c). These results highlight the importance of non-maternal bacterial transmission routes and the complexity of MS transmission in young children. MS transmission follows the general rule of transmission in infectious disease, specifically, that when close contact is present, MS from any source can infect children. In the early life of infancy, MS transmission may mainly occur from family members and care-givers such as mothers, fathers, grandparents, and siblings. As the social contact of the child extends beyond their family, horizontal transmission from peer playmates becomes more prominent. Children that are placed in environments such as nurseries, daycare centers, or elementary schools for extended periods of time favor horizontal transmission from child to child. Therefore, prevention of MS transmission should be considered in a more broadened perspective and may be an effective path in prevention of caries microbiome dysbiosis in children and adults at high-risk for MS transmission. A summary of transmission routes is illustrated in Figure 3.

Erythritol and Xylitol in Caries Prevention

Since sugar intake plays a significant role in caries induction and dysbiosis of the microbiome, sugar substitutes, especially polyols, can play significant roles in combating dental caries by reducing acid production, EPS formation and plaque accumulation and therefore modify caries microbiome dysbiosis.

Current approaches of using polyols for caries prevention focus on topical uses such as chewing gum, lozenges and wipes with evidence of the anti-cariogenic effects of chewing sorbitol, xylitol or sorbitol/xylitol gum (Mickenautsch et al. 2007). Xylitol is the most studied polyol with moderate evidence on the anti-caries effect of topical xylitol use over 4 grams daily compared to other polyols or fluoride varnish (Janakiram et al. 2017). Recent studies also demonstrated a better anti-caries effect of erythritol, a non-nutritive 4-carbon polyol with similar sweetness to sucrose, less laxative side-effects compared to other polyols, and potential cardiovascular benefits especially to diabetic patients, than xylitol/sorbitol (de Cock et al. 2016).

The mechanisms of xylitol/erythritol anti-caries activity include decreased dental plaque formation and reduced adherence of common oral streptococci, inhibited growth of cariogenic bacteria and GTF activities, and decreased expression of bacterial genes involved in sucrose metabolism (de Cock et al. 2016; Milgrom et al. 2012; Zhan et al. 2012b). Short-term (six-months or less) daily-xylitol-dose over 6g has been reported to reduce oral MS levels (Holgerson et al. 2007), while long-term (over 12-months) xylitol-use on MS showed mixed results (Zhan et al. 2012a). Habitual-use of xylitol can induce xylitol-resistant MS. It was hypothesized that long-term xylitol-use may select for xylitol-resistant MS with less virulence, leading to unchanged MS levels in some studies (Soderling 2009). However, the effects of xylitol on MS virulence using xylitol-resistant or sensitive MS have shown conflicting results on their cariogenicity (Zhan et al. 2012b).

An important anti-caries effect of xylitol is that it prevents MS transmission/colonization in early childhood with a long-term impact on the oral microbiome and anti-caries effects in children (Soderling 2009). As MS transmission has multiple sources, prevention of MS transmission may be more effective if it is child-centered. We explored the effect of direct use of xylitol-wipes in high-risk infants on their MS colonization and found it significantly reduced caries formation in the infants but failed to prevent MS colonization in this population (Zhan et al. 2012a; Zhan et al. 2012b). In the light that xylitol also did not reduce the MS levels in mothers but reduced the MS transmission to children (Soderling 2009), xylitol use may be effective in altering MS colonization or virulence rather than by reduction of MS levels. Our study indicated that xylitol-wipe use in children altered the stability of MS colonization but showed no impact on xylitol tolerance, acid production and biofilm formation of MS (Zhan et al. 2012b). Since mutacin activity may affect MS transmission (Zhan et al. 2012c), we also investigated the mutacin gene distribution in MS through high-throughput Illumina sequencing of the MS isolated from the xylitol-wipe study. Fifteen biosynthetic gene clusters were identified that account for the majority of variation in the MS genome and may have mutacin activity. However, no statistically significant differences were found in mutacin gene distribution between MS isolated from the xylitol *vs* the placebo groups (unpublished data). Because the etiology of dental caries is microbiome dysbiosis, future studies on anti-caries mechanisms against bacteria transmission should include other cariogenic and commensal bacteria, microbiome changes, and bacterial virulence factors in both mothers and children. There are no studies that investigate the potential of erythritol to prevent bacterial transmission and caries formation.

In addition, there is new insight into the linkage of sugar consumption with systemic noninfectious diseases including obesity, diabetes, hypertension and cardiovascular diseases (WHO 2015). As a main source for natural sugar substitutes, polyols, especially xylitol and erythritol use, hold a unique position to have not only oral benefits but also overall health benefits with respect to the non-infectious diseases listed above. Future dental studies should also include measurements for these systemic diseases.

Conclusions

Dental caries is a disease that esults from microbiome dysbiosis with involvement of multiple cariogenic species, including MS, lactobacilli, as well as *Scardovia wiggsiae* in the *Actinomyces/Bifidobacterium* family and several *Actinomyces* species that have the cariogenic traits of acid production and acid tolerance. It is hypothesized that selected putative cariogenic species may be involved in more aggressive dental caries while extended multiple species determinations will be warranted to determine the caries-profile of the population and/or individuals under study. Therefore, anti-caries treatment may be more logical in focusing on correcting the microbiome dysbiosis than eliminating a single pathological agent.

Sugar intake plays an important role, leading to microbiome dysbiosis, thereby determining the fate of caries development. The MS transmission involves multiple mechanisms and can have long-term impacts on the oral microbiome. Intervention in cariogenic bacteria transmission in early childhood may provide effective long-term caries prevention. Xylitol and erythritol may also serve as good candidates as part of anti-caries regimens via their roles as sugar substitutes, oral microbiome modifiers, and agents to prevent cariogenic bacteria transmission with both oral and systemic benefits in early childhood. Additional studies are needed to further elucidate the mechanisms of action of xylitol and erythritol with respect to microbiome shifts and bacterial virulence in addition to cariogenic bacteria levels, as well as their benefits for overall health.

The anti-caries agent, C16G2, targeting the most studied cariogenic bacteria *S. mutans*, have shown good safety for short-term oral topical use and promising effects in reducing *S. mutans in vitro* and *in vivo* with the simultaneous promotion of oral commensal bacteria. Future study on its anti-caries effect will need to include its long-term impact on the oral microbiome and effects on other important cariogenic bacteria.

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We declare that the research project upon which our manuscript is based, is (i) original, (ii) not presently under consideration for publication elsewhere, (iii) free of conflict of interest (e.g., not edited by the funding agency or organization), and (iv) conducted by the highest principles of human subject studies.

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Figure 1. The caries process according to an extended caries ecological hypothesis (Takahashi and Nyvad, 2011).

Figure 2. AP-PCR results: An example of one triad with mutans streptococci (MS) transmission, using OPA-5 primer. Lanes 1 to 10 show isolates from the child, lanes 11 to 20 are isolates from the mother, and lanes 21 to 30 are isolates from the grandmother. (*) indicates a shared genotype between the grandmother and the child (transmission). (◊) indicates another uniquely shared genotype between the grandmother, the mother, and the child.

Figure 3. Routes of mutans streptococci (MS) transmission. Vertical transmission of MS refers to MS transmitted from the mother, the father or other caregivers to a child. Horizontal transmission refers to MS transmitted from siblings, intimate playmates or close partners or spouses to a child or an adult.

Table 1. Distribution of sources of mutans streptococci transmission among the grandmother-mother-child triads