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Role of sweeteners in the etiology and prevention of dental caries*

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Abstract: Dental caries is a multifactorial disease that is caused by an interplay of three major factors, i.e., teeth, cariogenic bacteria, and fermentable sugars. *Streptococcus mutans* and *S. sobrinus*, collectively termed mutans streptococci (MS), are principal causative agents of dental caries. Initial MS-tooth surface attachment is followed by firm and irreversible adhesion of MS to the tooth surface, accompanied by the synthesis of water-insoluble glucan from sucrose via enzymatic action of glucosyltransferases (GTases). MS induce severe dental caries in rats fed on a high-sucrose diet. Epidemiological surveys indicate that frequent sucrose intakes are associated with high prevalence of dental caries in humans. In contrast, dietary sucrose restrictions and/or use of nonfermentable sucrose substitutes clearly influence the GTase activities of MS, resulting in decreased caries development. Structural isomers of sucrose (i.e., disaccharides composed of glucose and fructose with different linkages) will not function as substrates for GTases of MS, nor be utilized as energy sources by MS. Palatinose and trehalulose are included in this category, and are produced in commercial scales in Japan. Glucose oligomers containing α-1, 6 and/or α-1, 4 linkages are found to inhibit glucan synthesis by MS from sucrose, although these oligomers are hydrolyzed by MS to release acids. Lastly, sugar alcohols, including maltitol and palatinit, are useful as non-caries-inducing sweeteners.

INTRODUCTION

Dental caries has been recognized as the chronic local destruction of teeth by the activity of oral bacteria [1–3]. The initial lesion of dental caries (i.e., demineralized white spot of tooth enamel) is caused by the acid produced by the fermentation of dietary carbohydrates. Earlier, it was believed that a diet of some carbohydrates may have a role in the development of dental caries. Since the 19th century, when sucrose became a daily used sweetener by many people worldwide, the increasing prevalence of dental caries had also been noticed. However, the associations between caries development and high-sucrose diets in children had not been proved scientifically, until the epidemiological surveys revealed the association. Scientists working in this field have been fascinated by the epoch-making discovery of the tubercle bacillus (i.e., *Mycobacterium tuberculosis*) by Robert Koch in 1882 and fervently looked for the cause of dental caries. Under the influence of Koch, W. D. Miller, who worked in Berlin, sought the etiology of caries based on the bacteriological approach. He reached the conclusion that dental caries was caused by bacteria that fermented carbohydrates in foods and produced acids to destroy the hard tissues of teeth. His chemico-parasitic theory was supported by many of his contemporaries and was further strengthened by his successors. Although his theory agrees on many points with the current con-

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cept shown later, there were some insufficiencies in some important issues. Miller stressed the importance of acidogenic microbes as caries-inducing organisms, and he and his successors believed until the mid-1950s that oral lactobacilli should be cariogenic organisms [1–3].

Today, however, it is known that lactobacilli per se do not induce dental caries, although they are secondary invaders in caries lesions [1]. Instead, some selected species of oral streptococci have been demonstrated to be cariogenic in experimental animals. We call these cariogenic streptococci mutans streptococci (MS). MS include several different species; *Streptococcus mutans* and *S. sobrinus*, which are found in human caries, while other species such as *S. rattus*, *S. cricetus*, and some others are found in the carious lesions of experimental and wild animals [4]. Ample evidence indicates an etiological relationship between caries development and MS in humans [5].

In this short review, the focus is placed on the close association among MS, sucrose, and caries development [6,7]. Conversely, the interception of this association may lead to a decrease of or even an inhibition of caries development.

VIRULENCE TRAITS OF MS

Oral flora is a complex ecosystem with a wide variety of bacterial species [8]. Dental plaque develops on the enamel surface that is coated by a pellicle composed primarily of salivary proteins. The number of bacteria in dental plaque can reach ~10^8 per mg (wet weight). Streptococcal species occupy approximately 1/3 of the total viable organisms of plaque [3]. In addition to MS, several streptococcal species are frequently found in the human oral cavity. Among these are *Streptococcus sanguis*, *S. gordonii*, *S. oralis*, *S. mitis*, *S. salivarius*, and others [9]. It has been shown that these oral streptococcal species are not involved in the development of dental caries in experimental animals. However, it should be noted that all these species are highly acidogenic when sucrose, glucose, or fructose is given [10].

Biochemical comparison of MS with other species gave a clue to identify virulence-related traits. In the initial phase of studies, MS were found to possess the ability to adhere to the glass surface when they are grown in sucrose-containing culture medium. This property was subsequently ascribed to the enzymatic action of glucosyltransferases (GTases), which generate adhesive, water-insoluble glucans from sucrose [11,12]. The glucosidic linkage of sucrose contains high energy that is similar to that of ATP (~6.6 kcal/mol) [3]. Although other oral streptococci such as *S. sanguis*, *S. gordonii*, and *S. salivarius* can synthesize glucans and/or fructans from sucrose, these polysaccharides are usually not adhesive to glass surfaces. Therefore, the growing cells of these organisms in sucrose-containing broth do not adhere to the glass surface. However, bacterial polysaccharides are of great importance in the formation and ecology of dental plaque [13].

MS possess plural genes in their genome that encode the synthesis of GTase molecules [14]. In *S. mutans*, three GTase genes, *gtfB*, *gtfC*, and *gtfD* are known, and the former two genes encode GTase-I and GTase-SI which mainly synthesize water-insoluble glucans, and are found in a cell-associated form. The latter, on the other hand, encodes GTase-S synthesizing water-soluble glucan which is released into culture supernatant. Concerted action of these GTases is required for firm adhesion of the growing cells of *S. mutans*. Recent studies indicate that GTase-SI plays a key role in the adhesion of *S. mutans*. The nuleotide sequence of *S. mutans* MT8148 *gtf* genes suggested that the *gtfC* gene located immediately downstream to the *gtfB* gene. Considerable homology was found between the deduced amino acid sequences of the GTase-I and GTase-SI [15]. In this regard, *S. sobrinus* possesses 4 *gtf* genes [14].

MS synthesize glucans from sucrose, and free fructose is released in this reaction. This fructose and other common sugars present in daily foods are utilized as energy sources for MS and other bacteria in dental plaque. They produce a large quantity of acids such as lactic acid from fermentable sugars. If the pH falls below 5.6 on the tooth surface, decalcification of enamel or even dentin of the tooth may occur. This decalcification is induced with acids produced by the surface-adhered MS that synthesize water-insoluble glucan from sucrose. Acids are entrapped between the tooth surface and adhered MS, and a drop in pH should easily occur in the localized area [3,16].

SUCROSE AND SUCROSE SUBSTITUTES

Criminal sugar

Sucrose is cheap, easily produced from sugar cane or sugar beet, and high in calories. Sucrose, as well as other sugars obtained from potatoes, rice, wheat, etc., highly contribute to diets and processed foods as a sweetener, bulking mass, preservative, moistener, texture modifier, etc. There has been a broad consensus that quantity and frequency of consumption of sucrose-containing foods has a significance on caries incidence [17]. Statistics in Japan during World War II indicated that the import of sucrose was drastically obstructed, and per capita consumption of sucrose decreased to 0.2 kg/year in 1946 from 16.7 kg/year in 1939. With the drop of sucrose consumption, caries prevalence in school children significantly decreased. As the import of sucrose was resumed after the War in the early 1950s, caries incidence became greater than the pre-war levels [18]. Similar statistics were seen in the United Kingdom and Scandinavian countries. These findings during war-time life strongly suggest that caries development can be strongly influenced by dietary factors [3].

More controlled studies on caries development in humans were done in Vipeholm hospital for mentally retarded persons in Sweden, during 1946–1951 [19]. Adult inpatients were followed on a nutritionally adequate diet and showed little increase in caries. However, when groups of patients were given sweets as the form of coffee or caramel between meals, caries development increased sharply (× 10 fold).

Another supporting finding on the effect of sucrose-containing diets emerged from the study of Hopewood House, a children's home, in Australia [20]. A population of 80 children were brought up with diets excluding sugars and refined carbohydrates. Children ages 5–13 showed 10 % of the caries found in the control group of public school children near the home. However, a marked increase in caries incidence was observed in the children when they were discharged from the home and were fed ordinary foods with sucrose and other refined carbohydrates.

Dental caries can be induced experimentally in MS-infected animals, especially in rodents, including rats or hamsters, which have been conveniently used for objective cariogenicity testing of sugars and various foods [10]. The assessment of the caries potential using animals is very valuable to food manufacturers as well as to consumers/patients/children to guide their dietary habits for their oral health. Sucrose was found to give the highest caries score as compared to other fermentable sugars [21].

Search for better sucrose substitutes

Sucrose has many excellent properties as a sweetening sugar. Its manufacturing technology is fully developed and low in cost [22]. The only and the greatest disadvantage of sucrose is that it is an efficient enhancer of the virulence factors of MS. Thus, the search for ideal sugar substitutes has become a high priority [3,16]. The ideal sucrose substitute should have the following properties:

- It should not be used as an energy source by MS and other oral bacteria so that no significant production of acids by these organisms will be followed.
- It should not act as the substrate for the synthesis of glucan by GTases of MS, however it should inhibit glucan synthesis from sucrose.
- It should have good taste.
- After taking orally, it should be degraded properly by the intestinal digestive enzymes and/or microorganisms and it should have no side-effect on the body.

STRUCTURAL ISOMERS OF SUCROSE

The chemical structure of sucrose is α-D-glucopyranosyl-1,2-β-D-fructofuranoside. There are different structural isomers of sucrose composed of glucose and fructose of which glucosidic linkages are

α-1, 1 (trehalulose), α-1, 3 (turanose), α-1, 4 (maltulose), α-1, 5 (leucrose), and α-1, 6 (palatinose, also referred to isomaltulose). These analogs are found in honey, beet extract, and other natural products in low concentrations [23,24]. Among these, palatinose is the sucrose analog that has been most extensively studied.

Palatinose

Palatinose is produced from sucrose by the enzymatic action of a cell-associated glucosyltransferase of *Protaminobacter rubrum* when grown in sucrose-containing broth [25]. Whole *P. rubrum* cells are entrapped in an alginate gel and used as an immobilized enzyme for large-scale column chromatographic production of palatinose [26,27]. The column effluent typically contains palatinose, trehalulose, and other components at a ratio of 86:9:5. Palatinose can be purified into crystalline form by ion exchange chromatography, followed by de-salting and concentration. Crystalline palatinose thus obtained contains equal molar of crystalline water. Uncrystallizable syrup also contains high concentrations of trehalulose and palatinose and is used as a sweetening agent [28].

The relative sweetness of palatinose is ~42 % of sucrose. Palatinose is very stable with low hygroscopicity and high melting temperature, and is resistant to acid hydrolysis as compared to sucrose. The reducing power is about half that of glucose [28].

Maillard reaction occurs when palatinose solution is heated at >140 °C, especially in the presence of amino acids/peptides. Palatinose is digested slowly in the intestine by the enzymatic action of isomaltase. There is a prolonged and low increase in the concentration of blood sugar after ingestion of palatinose. The oral intake of palatinose does not induce diarrhea.

Palatinose and other sucrose isomers have been found not to be utilized by most oral bacteria. Strains of MS did not ferment palatinose significantly even after 48 h incubation of the organisms [28–30]. Furthermore, MS did not synthesize glucan from palatinose and other sucrose isomers. When increasing concentrations of palatinose or trehalulose were added, glucan synthesis by MS from sucrose was suppressed and cell adhesion to the glass surface was decreased accordingly. In addition, and most importantly, specific pathogen-free (SPF) rats infected with *S. mutans* or *S. sobrinus* and fed a powdered diet containing 56 % palatinose did not develop significant dental caries [28]. In contrast, rats infected and fed on a 56 % sucrose-containing diet develop extensive caries, forming voluminous dental plaque accumulation on the molar teeth. Following these in vitro and in vivo studies, a human trial was done to examine the effect of palatinose, and it was found that the intake of between-meal snacks containing palatinose resulted in the lowest plaque index, whereas high-sucrose snacks induced a significantly greater plaque deposition [31]. Although pH drop below pH 6.1 in human dental plaque in vivo was not found with palatinose [32], frequent mouth rinsings with 50 % palatinose might induce a microbial adaptation to palatinose, and a significant pH drop (pH 6.1) was observed [33].

Very recently, Peltroche-Llacsahuanga et al. [34] reported that some strains belonging to *Stomatococcus mucilaginosus*, *Lactococcus lactis*, *Lactobacillus acidophilus*, *Streptococcus sanguis*, *S. oralis*, and *S. anginosus* did produce acid from palatinose, and these authors suspected that palatinose (as well as leucrose) might be cariogenic. However, the finding that prolonged (56 days) feeding of palatinose to rats, which harbors MS and lactobacilli in their flora, did not induce dental caries does not support this claim [28]. In this regard, it should be noted that *Klebsiella pneumoniae* has been shown to metabolize not only sucrose, but also its five linkage isomers [35].

Currently, palatinose and related isomers (mainly trehalulose) are commercially used as sweeteners in many between-meal food items in Japan.

Trehalulose

Trehalulose is a structural analog of sucrose, i.e., α-D-glucopyranosyl-1, 1-D-fructose, and is found in high concentrations in a noncrystallizable syrup that is a by-product of the production of palatinose

from sucrose. Trehalulose is purified by column chromatography to a purity of 98.5 %. It is chemically and physically stable, while being degraded by intestinal isomaltase to produce free glucose and fructose in the same way as palatinose [29,36].

Trehalulose does not serve as a substrate for GTases of MS to synthesize glucans, nor is it utilized as an energy source to produce acids. The relative sweetness of trehalulose is ca. 60 % that of sucrose. No diarrhea is induced by intake of this sucrose isomer. Like palatinose or wheat flour, trehalulose (56 %) induced no significant dental caries in SPF rats infected with either *S. mutans* or *S. sobrinus*, and only slight plaque accumulation was found on the molars. The replacement of 50 or 70 % of the sucrose content by trehalulose resulted in a significant reduction in caries score as compared to that of the group fed a 56 % sucrose diet [29].

Other sucrose isomers such as turanose, maltulose, and leucrose are known to be not utilized by MS and many other oral bacterial species to produce acids or to synthesize glucan by their GTases [30,37].

Recently, it has been reported that leucrose, in addition to palatinose, can be utilized by 11 bacterial strains out of 5 species. It appears, however, that many of these species are not etiologically related to dental caries, nor major members of the microbial flora on the tooth surface [34].

α-OLIGOSACCHARIDES

Glucan synthesis by MS from sucrose is known to be clearly inhibited by a variety of sugars containing α-glucosidic linkages such as maltose (α-1, 4), isomaltose (α-1, 6), xylosylfructose (α1-β2), or even trisaccharides, including panose (α-1, 6 and α-1, 4), isomaltotriose (α-1, 6 and α-1, 6) and maltotriose (α-1, 4 and α-1, 4). Oligosaccharides containing the α-1, 6 linkage are resistant to the hydrolysis by many bacterial species. PI-oligosaccharides (PIOS) containing high panose, maltose, and isomaltose are produced from corn starch. Starch is first hydrolyzed by the treatment of α-amylase, followed by β-amylase and transglucosidase to give a series of α-oligosaccharides with various degree of polymerization [38]. PIOS are mildly sweet and low in viscosity, and possess high moisture-retaining activity. Isomaltose, maltose, and panose are the major components of PIOS, and these sugars suppress glucan synthesis, owing to the transfer of the glucosyl moiety of sucrose to PIOS, and inhibit the cellular adhesion of MS to the glass surface [39]. Therefore, use of these α-oligosaccharides may suppress the dental caries induced by MS and sucrose. In the experimental model system, a PIOS mixture induced significant, but minimal dental caries in SPF rats infected with either *S. mutans* or *S. sobrinus*. The replacement of half of the dietary sucrose content with PIOS markedly reduced the caries levels as compared to the sucrose diet group of rats [40].

SUGAR ALCOHOLS

Sugars are converted into sugar alcohol when they are hydrogenated using specific catalyst in a high-temperature and high-pressure condition. Sugar alcohols show moderate sweetness and lower calories. However, when taken in excess, loosening of the bowel and diarrhea may occur frequently. They are stable under various pH conditions and high temperatures. Sugar alcohols are not usually utilized by oral microorganisms. Xylitol has been used widely as a sweetener of chewing gum, candies, etc. [41]. We have examined the usefulness of maltitol and palatinit (both are disaccharide alcohols produced from maltose and palatinose) as cariostatic sweeteners.

Maltitol

Maltitol is produced by hydrogenating maltose, and composed of glucose and sorbitol, linked with α-1, 4 linkage. Because of the presence of this α-glucosidic linkage, maltitol suppresses the glucan synthesis by MS from sucrose like maltose. However, maltitol is not utilized by MS and many other oral

bacterial species, and therefore, no pH drop was obtained when these strains are cultured in maltitol medium. It does not induce dental caries in SPF rats infected with either *S. mutans* or *S. sobrinus*. Furthermore, cofeeding of sucrose and maltitol decreased the cariogenic properties of sucrose in rats infected with *S. sobrinus*, although no such reduction in caries development was observed in rats infected with *S. mutans* [42].

It should be noted that diarrhea was found in all rats fed with a maltitol-containing diet. Maximum tolerable concentration of maltitol in the diet was ~20 % (w/w), which may be similar to other sugar alcohols. In humans, the maximum nonlaxative dose of maltitol is determined to be ~0.3 g/kg body weight [43]. The sweetness of maltitol is approximately 90 % of that of sucrose, which is higher than that of maltose. The cost of maltitol is considerably lower than xylitol.

Palatinit

Palatinit is produced by the hydrogenation of palatinose and is an equimolar mixture of α-D-glucopyranosyl-1,6-mannitol (GPM) and α-D-glucopyranosyl-1,6-sorbitol (GPS). The relative sweetness of palatinit is ~50 % of sucrose; however, its taste is similar to that of sucrose. Palatinit is resistant to enzymatatic degradation in the intestine, and not fermented by many bacterial species including MS. If taken in excess, it may cause diarrhea. The maximum tolerable single dose of palatinit appears to be ca. 35 g for Europeans and 15 to 20 g for Japanese [44,45].

Like maltitol, palatinit did not induce dental caries in SPF rats infected with *S. mutans* or *S. sobrinus* [46].

SUCROSE DERIVATIVE

Chemically modified sucrose derivatives have been examined for their usefulness as a noncariogenic sweetening agent. Hough and Khan [47] found that halogenated sucrose derivatives yield intensely sweet compounds. A compound named sucralose (i.e., 1′, 4′, 6′ trideoxy-trichloro-galactosucrose) was shown to possess intense sweetness, which is ca. 600 times sweeter than sucrose. It did not support the growth of many strains of oral bacterial species when used as a sole carbon source. The addition of sucralose in glucose agar medium resulted in the inhibition of growth of certain oral bacterial species including MS. Sucralose was found to inhibit the synthesis of glucan and fructan from sucrose by *S. sobrinus* GTase and *S. salivarius* FTase, respectively [48,49]. Furthermore, it has been claimed that sucralose, like sugar alcohols, may promote recalcification of the tooth surface affected by the carious attack [50]. Sucralose is stable even in an acidic condition, which is in contrast to aspartame, a widely used intense sweetener. Thus, sucralose is widely used for drinks as a substitute for sucrose, but not as a bulk agent. Sucralose is not absorbed easily to the alimentary canal and is eliminated in the urine [49].

CONCLUSION

High levels of sucrose consumption and frequent intakes of sucrose-containing between-meals snacks are critical risk factors for children with MS in their oral flora. Intervention of the risk factors by sucrose substitutes should be a rational approach to prevent dental caries in many countries.

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