

Dental caries is a preventable infectious disease

Mayooran Balakrishnan,* Robin S. Simmonds,† John R Tagg†

Abstract

Dental caries is the most common infectious disease affecting humans. The principal causative agents are a group of streptococcal species collectively referred to as the mutans streptococci of which *Streptococcus mutans* and *Streptococcus sobrinus* are the most important agents of human caries. This review outlines what is currently known about these ubiquitous pathogens and discusses novel methods for elimination of these bacteria from dental plaque.

Key words: Dental caries, mutans streptococcus, vaccines, chemoprophylactic agents, bacteriocins.

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Introduction

Dental caries is the predominant cause of tooth loss in children and young adults. Although the disease most commonly affects the crown of the tooth, caries of the tooth root is also prevalent, especially in older populations. Caries of the crown of the tooth initially presents as a white spot in the enamel and on the root as soft areas in the cementum and dentine. As caries progresses, more extensive destruction of the enamel and dentine occurs, followed by inflammation of the pulp and periapical tissues.

In recent years, the prevalence of dental caries in most western countries has steadily declined. By contrast, studies done in some developing countries such as Zambia, Indonesia, Sudan, Nigeria and Thailand have indicated a marked increase in dental caries.¹

The non-specific plaque hypothesis and dental caries

In the 16th century, suggestions of the possible involvement of micro-organisms in dental caries

were made by Antonie van Leeuwenhoek, who first saw plaque bacteria under the microscope. Following this, several other early investigators also suggested a possible causal association of micro-organisms with this disease. In the late 1800s, Miller proposed the chemicoparasitic theory of caries development. According to Miller, micro-organisms in the oral cavity caused the breakdown of dietary carbohydrates due to the activity of enzymes they produced and this in turn led to acid production and enamel demineralization. Miller considered that all bacteria in the mouth were potentially cariogenic, a concept now known as the 'non-specific plaque hypothesis'.²

The specific plaque hypothesis and dental caries

The first report of the involvement of streptococci in the aetiology of dental caries was by Clarke³ in 1924. From human carious lesions, Clarke isolated streptococci with distinctive characteristics and named them *Streptococcus mutans*. However, direct evidence for the involvement of specific micro-organisms in dental caries first came from the studies of Keyes⁴ in 1960. Keyes observed that albino hamsters only developed caries when caged together with 'caries-active' hamsters. Keyes also found that caries-active dams became caries-inactive when treated with antibiotics such as penicillin and erythromycin. This study concluded that, even though the albino hamsters harboured complex bacterial populations, they did not develop caries because they did not harbour cariogenic bacteria. However, they did develop caries when they acquired cariogenic organisms from caries-active hamsters. It was now evident that specific micro-organisms were involved in the induction of dental caries and that the disease was transmissible.

More recent studies have shown the bacteria previously referred to as *S.mutans* are now subdivisible into seven distinct species.⁵ These species are often collectively referred to as the mutans streptococci (MS) because they have a number of common properties relevant to caries-inducing

*Department of Oral Sciences and Orthodontics, University of Otago, Dunedin, New Zealand.

†Department of Microbiology, University of Otago, Dunedin, New Zealand.

ability. In addition, all these species are found in the oral cavity and it is more convenient to refer to them as a cluster rather than as individual species. It is now widely agreed that MS are the principal aetiological agents of dental caries. This involvement of specific bacteria in caries development is referred to as the 'specific plaque hypothesis'.²

Mutans streptococci and dental caries

Animal experiments

Numerous studies have shown MS can bring about caries in pits and fissures as well as on smooth, approximal and root surfaces of the teeth of both gnotobiotic and conventional animals. Moreover, the caries induced by MS is more severe than that caused by other streptococci.⁶ Further evidence for MS involvement in the aetiology of caries has come from immunization studies. In one such study, the oral administration of *S.mutans* cells to gnotobiotic rats induced the production of secretory antibodies in the saliva and this correlated with a reduction in caries incidence in these animals.⁷ In another study, intravenous administration of *S.mutans* cells to monkeys led to a serum antibody response and an associated decrease in caries incidence.⁸

Human studies

Evidence for the association of MS with dental caries in humans has come from epidemiological studies. These have shown that populations with a high caries incidence have relatively higher levels of MS than populations with a low incidence of caries.⁶ Similarly, caries reduction in Swedish children was paralleled by a reduction in salivary counts of MS.⁹ Reduction of MS in pregnant women led to reduced colonization and concomitantly reduced caries in their children.¹⁰ Many studies have shown MS are regularly isolated from incipient or well developed carious lesions, but less commonly from sound tooth surfaces.⁶ In a longitudinal study, it was found MS levels in plaque increased 6-24 months before the clinical appearance of dental caries.¹¹ Increases in the proportion of MS were observed to occur before root caries lesions developed or when these lesions became active.¹² However, it should be noted that sound enamel may sometimes be colonized with a relatively high number of MS¹¹ and that some caries-free populations have high plaque counts of MS.¹³ In these situations, caries may not have developed either because the teeth are relatively resistant to acid attack or because the individuals did not have a particularly cariogenic diet.

Immunological studies have also implicated MS in the aetiology of dental caries in humans. In general, high titres of salivary and serum antibodies to MS antigens have been found in low caries populations and the prevalence of caries is high in

immunocompromised patients.¹⁴ Studies using broadly active plaque-control agents such as chlorhexidine provide further evidence of the association of MS and dental caries in that regular application can lead to a significant reduction in the levels of both MS and caries incidence.¹⁵

Non-mutans streptococci and dental caries

For some time it was thought that lactobacilli were the primary aetiological agents of dental caries because these bacteria produce large amounts of acid in the presence of sugars and are able to survive at very low pH values. Some lactobacilli were shown capable of inducing caries in experimental animals.⁶ However, lactobacilli generally have a relatively low affinity for tooth surfaces and do not accumulate in large numbers within plaque. They mainly colonize the oral mucosa.⁶ Although lactobacilli could not be detected over white spot lesions,⁶ they were present in 85 per cent of progressive lesions.¹⁶ From these observations, it is now believed lactobacilli probably play a more important role in the progression of dental caries rather than in initiation of the disease.

Various studies have shown that certain strains of enterococci and other oral streptococcal species including *S.sanguis*, *S.oralis*, *S.mitis* and *S.salivarius* are capable of causing caries development in rats. Formation of fissure caries, rather than smooth surface lesions, was most evident and the severity of disease was mild compared with that induced by MS.^{6,17} On the basis of these findings, the contribution of non-MS to the aetiology of dental caries appears minimal. The accumulated evidence from animal experiments and human epidemiological studies overwhelmingly indicates MS are the principal aetiological agents of both enamel and root caries.

Different species within mutans streptococci

MS have been divided into eight serotypes (a-h)¹⁸⁻²⁰ based on the detection of specific carbohydrate antigens in the cell wall. For serotypes a, d and g, the antigens are composed of glucose, galactose and rhamnose; for serotypes c, e and f, the antigens comprise glucose and rhamnose; and the antigens for serotype b are galactose and rhamnose.⁵ MS can also be classified into different biotypes based upon sugar fermentation reactions.²¹

DNA-DNA hybridization studies indicated the existence of at least four distinct genetic groups²² and subsequent studies led to further groups being added. Presently seven different MS species – known as *S.mutans* (serotypes c, e and f), *S.rattus* (serotype b), *S.cricetus* (serotype a), *S.sobrinus* (serotypes d and g), *S.ferus* (serotype c),²³ *S.macacae* (serotype c)²⁴ and *S.downei* (serotype h) – are recognized.²⁵

Distribution and transmission of *S.mutans*

MS are found mainly in dental plaque. Molar teeth are more heavily colonized than anterior teeth and fissures in these teeth are more susceptible to colonization than approximal, buccal or lingual surfaces. Also, except for the occlusal surfaces, teeth with restorations harbour more MS than those with sound surfaces.²⁶ Infants acquire MS soon after their teeth erupt.²⁷ After extraction of the teeth, MS disappear, but reappear upon use of dentures.²⁸ MS may also be isolated from pre-dentate infants who wear obturators.²⁹ These observations suggest hard surfaces are essential for MS colonization.

MS first colonize infants' teeth from 19-31 months of age, a period described by Caufield et al²⁷ as the 'window of infectivity'. It has been suggested if infants fail to acquire MS during this period, they are unlikely to be colonized until around 6 years of age when the permanent molar teeth begin to erupt.²⁷ Colonization by MS increases with age²⁷ and with the increased presence of rough surfaces such as enamel hypoplastic lesions.³⁰ Serotyping, bacteriocin typing and genetic studies have shown that specific MS strains are commonly transferred from a mother to her infant.^{27,31} Saliva is a vehicle for the transfer of these bacteria and exchange can occur through kissing or via saliva-contaminated food. It has been reported if the level of MS in the mother's saliva is in excess of 10⁶/ml, there is a 70 per cent chance the infant will acquire MS from the mother within three years. By contrast, if the level of MS in the mother's saliva is less than 3×10⁵/ml, the chance the infant will acquire MS from the mother within three years is reduced to 20 per cent.¹⁰

Streptococcus mutans and *S.sobrinus* are the MS most commonly found in humans. Their distribution is world-wide, with *S.mutans* the more frequently isolated.³² *Streptococcus sobrinus* is generally found in association with *S.mutans* and is thought to be principally responsible for the development of smooth surface caries.³³ *Streptococcus rattus*, although first isolated from rats,⁵ has also been detected in specimens from humans, most commonly African populations.³⁴ *Streptococcus cricetus* was first recovered from hamsters⁵ but has also been isolated from humans.³² *Streptococcus ferus* initially came from wild rats and *S.macacae* and *S.downei* were isolated from monkeys.⁵ There are no reported isolations of the latter three species from humans.

Cariogenic attributes of mutans streptococci

MS possess certain properties that enable them to predominate in dental plaque and induce caries development. These include extracellular polysaccharide synthesis, acidogenicity (acid production), aciduricity (ability to survive in an acidic environment), intracellular polysaccharide synthesis and endodextranase production.

Extracellular polysaccharide synthesis and adhesion

Adhesion of MS to the teeth occurs as a two step process. Initial attachment is reversible and mediated by surface components of the MS.³⁵ Functional domains within MS fimbriae recognize and bind to components of the acquired enamel pellicle. Reversible attachment is followed by sucrose-dependent irreversible attachment. In the presence of sucrose, MS synthesize both water-soluble (predominantly α1-6 linked) and water-insoluble (predominantly α1-3 linked) polyglucose molecules referred to as glucans. Various glucosyltransferases are involved in this glucan synthesis. Some of the newly synthesized glucan remains bound to the glucosyltransferase, which itself may be attached to the cell surface. Glucan can also bind to a protein (glucan binding protein) on the cell surface.¹⁴ Only sucrose can be utilized in the production of these extracellular polysaccharides. Various studies have shown that, in comparison with their parent strains, mutants that are unable to synthesize water-insoluble glucan have a decreased ability to adhere to the teeth and induce a lower rate of caries in experimental animals.^{36,37} These experiments indicate the water-insoluble glucan is an important virulence factor.

Acidogenicity

MS can ferment various sugars to produce lactic acid. Lactate dehydrogenase (LDH) enzymes convert propionate to lactate, but when carbohydrate is limited these bacteria produce formate, acetate and ethanol. It seems lactic acid is the most important acid involved in the aetiology of dental caries because it is the strongest acid produced in large quantities by the MS. Dietary sugars other than sucrose, for example glucose and lactose, can also induce caries formation. However, these sugars are less cariogenic than sucrose, because, in addition to being converted to acidic metabolites, sucrose is also uniquely utilized for extracellular polysaccharide synthesis. Starch is less cariogenic than other dietary sugars because it does not readily diffuse into plaque and is less readily hydrolyzed.³⁸ It has been demonstrated that *S.sobrinus* is more acidogenic³⁹ and more highly cariogenic than the other MS.³⁹

Aciduricity

MS can grow at low pH values, some strains even growing at less than pH 4. These streptococci produce large amounts of a membrane-associated ATPase, capable of functioning at low pH, which helps to pump H⁺ ions from the cell and thus reduce intracellular acidification.⁴⁰

Intracellular polysaccharide synthesis

In the presence of carbohydrates (not necessarily sucrose) some MS synthesize intracellular

polysaccharides (ICP) which typically resemble glycogen.⁴¹ When there is no exogenous carbohydrate, ICP can be metabolized leading to continued acid production. *Streptococcus sobrinus* does not synthesize ICP. Studies have shown that mutants of *S. mutans* which lack the ability to synthesize ICP, although still able to colonize the teeth of rats, are relatively less cariogenic than the parent strain.⁴²

Endodextranase production

MS produce endodextranases that cleave α 1-6 linkages within extracellular dextrans. *Streptococcus sanguis* and *S. mitis* colonize the teeth before mutans streptococci and form dextrans (glucans) rich in α 1-6 linkages. Endodextranases produced by MS assist the bacterium in its invasion of dextran-containing early dental plaque. Although endodextranase-negative mutants of *S. mutans* and *S. sobrinus* were able to cause caries development in mono-infected gnotobiotic rats, they proved non-cariogenic in conventional rats.⁴³

Current concepts of the pathogenesis of dental caries

MS, dietary sugars (especially sucrose) and a susceptible tooth surface are the important factors involved in the development of caries. Interaction between the cariogenic bacteria and sucrose leads to lactic acid production.² If lactic acid complexes with the calcium present in hydroxyapatite crystals on the surface of a tooth, it can cause demineralization. Other organic acids do not complex with calcium to the same extent and so do not promote the same degree of demineralization as lactic acid. Acids formed by bacteria will be neutralized by bicarbonate ions and by the peptide sialin present in saliva.² pH values below 5 are critical for enamel demineralization. If there is frequent exposure to sugar, the rate of demineralization of the tooth will exceed that of remineralization and caries will occur. If exposure to sugar is limited, the rate of remineralization of the tooth will exceed that of demineralization and caries development will be arrested.²

Prevention of dental caries

Dental caries can potentially be prevented by interfering with transmission of MS, eliminating established MS populations from the oral cavity, increasing the acid-resistance of the teeth and control of the carbohydrate composition of the diet. Although the possibilities listed above will be discussed separately, it is not intended to imply that a combination of methods cannot be used to prevent caries; indeed, the authors are of the opinion that optimal prevention of caries is probably best achieved through use of such combinations.

Interfering with transmission of MS

Vaccines

Vaccines have been spectacularly successful in protecting entire populations against infectious disease and their application to caries prevention would seem logical and timely. Researchers have taken three broad approaches to the development of anti-caries vaccines: oral, systemic and passive immunization. Either MS cells or purified MS antigens such as glucosyltransferases, antigen I/II (SpaA) and antigen A have been used as vaccines.⁴⁴ In experiments with animals and humans, administration of these antigens as either oral or systemic vaccines produced reductions in the number of MS.^{44,45} Unfortunately, during animal experiments, it was found systemic immunization with antigen I/II preparations resulted in the development of IgG antibodies that were cross-reactive with heart and kidney tissue, ruling out the use of such preparations in humans.⁴⁴ Oral administration led to the generation of an IgA-mediated immune response. In one study, it was found the ingestion of capsules containing killed *S. mutans* cells induced the production in saliva, milk and tears of IgA antibodies that were reactive against both glucosyltransferase and antigen I/II. In saliva, the increased concentration of IgA correlated with a 90-99 per cent reduction in the numbers of MS.⁴⁶ Oral immunization did not lead to the development of serum antibody responses, minimizing the risk of heart and kidney damage. In other studies it was shown that rats immunized with anti-idiotypic antibodies-bearing epitopes corresponding to MS antigens had reduced numbers of MS.⁴⁷

Passive immunization experiments, such as the application of monoclonal antibodies specific for antigen I/II to the teeth,⁴⁸ or the use of anti-mutans antibodies (predominantly IgG) derived from bovine milk, have brought about a reduction in the numbers of MS in animals and humans.⁴⁹ Other novel approaches include the expression of the *S. mutans* antigens glucosyltransferase A and SpaA in non-virulent salmonella, and the use of these bacteria as an oral vaccine to evoke a salivary IgA response in mice.⁵⁰ It was suggested that, by using this approach, a multivalent vaccine effective against both MS and other infective organisms could be developed.⁴⁴ A salivary IgA response against *S. mutans* was obtained in mice inoculated with antigen I/II coupled to cholera toxin sub-unit B via either peroral⁵¹ or intranasal⁵² routes. In another study, strong salivary IgA and serum IgG responses were induced in mice following the oral administration of recombinant *Lactococcus lactis* carrying a gene encoding a surface protein antigen of *S. mutans*.⁵³

In other recent developments, it has been reported that genes encoding both the heavy and light chains

of a murine monoclonal antibody (Guy's 13) had been cloned and expressed (Guy's 13 antibody) in *Nicotina tabacum*.⁵⁴ This antibody, which was directed against a cell surface adhesion, prevented the implantation of a *S. mutans* strain in human volunteers. More recently, Ma et al reported the application of extracts of transgenic plants expressing secretory IgA (consisting of Guy's 13 variable and gamma-constant domains coupled to human alpha chain constant domains) to the teeth of human volunteers prevented their recolonization by *S. mutans* for at least four months.⁵⁵ In general, passive immunization requires application of large quantities of antibodies and the use of transgenic plants is one means of preparing adequate amounts of secretory IgA. Potentially, given adequate expression levels, such antibodies could be administered through the dietary consumption of transgenic plants, eliminating the need to purify the antibodies before application.

Inhibition of glucan-mediated adhesion

In addition to reducing the sucrose content of the diet, glucan-mediated adhesion of MS can be minimized by substituting a structural analogue of sucrose for dietary sucrose. These analogues include the 6-amino derivatives of sucrose, acarbose, 6-deoxysucrose, deoxynojirimycin and ribocitrin, all of which are able to compete with sucrose for the active site of glucosyltransferases.⁴⁵ Inhibitors of glucosyltransferase also include molecules unrelated to sucrose, such as ellagic acid from *Geranium nepalense* and mutastein from *Aspergillus terreus*.⁴⁵

Glucan-hydrolyzing enzymes, including the dextranases which hydrolyse α 1-6 linkages and the mutanases which hydrolyse α 1-3 linkages, have been investigated for their ability to degrade glucan and hence reduce caries. Fitzgerald et al observed a significant reduction of coronal plaque in molars of albino hamsters when these animals were fed labchow or drinking water containing dextranase.⁵⁶ The erupted third molars of dextranase-treated animals were free of caries. By contrast, Guggenheim et al reported dextranase-containing labchow was not effective in protecting rats from caries.⁵⁷ Caldwell et al found dextranase-containing mouthwashes administered to humans did not have any significant effect on plaque score or on plaque dry weight.⁵⁸ This failure may have been because the dextranase was in contact with plaque for too short a time.⁵⁸ The authors suggested favourable results might be obtained by use of a more active preparation. To obtain maximum effect, the dextranase should be present in the mouth during the period of new dextran formation.⁵⁸ Lobene found a significant reduction of plaque dry weight in volunteers treated with dextranase mouthwashes, although the total

plaque surface area did not reduce significantly. No pathological lesions or irritation of the oral mucous membranes were observed with use of this mouthwash and there were no complaints about bad taste.⁵⁹ Generally, the overall effectiveness of dextranase treatments seems minimal and this is probably a reflection of the much higher proportion of α 1-3 than α 1-6 linked glucans present in plaque.⁵⁹

When it was realized the dental plaque matrix could be composed of high concentrations of mutan (having a high proportion of α 1-3 linked glucose residues), attention focused on the study of enzymes that could cleave α 1-3 linkages.⁶⁰ Guggenheim et al found a significant reduction of caries occurred in rats fed labchow containing mutanase (α 1-3 glucan 3-glucanohydrolase) whereas no significant caries reduction was observed in rats fed labchow containing dextranase (α 1-6 glucan 6-glucanohydrolase).⁶¹ In followup studies, Guggenheim et al reported the topical application of crude or partially purified mutanase reduced dental caries development in rats.⁶² The reduction of smooth surface and fissure caries brought about by this enzyme was as great as that obtained using chlorhexidine or fluoride.⁶² Kelstrup et al found that although the use of mutanase-containing chewing-gum by dental students reduced plaque accumulation, there was no significant difference in its bacterial composition. Also in this study, some side-effects including aphthous ulcers, soreness and bleeding of the tongue and disturbance of taste were observed.⁶⁰ Inoue et al found that rinsing the mouth with a purified mutanase preparation significantly reduced dental plaque formation in dental students.⁶³ Most of these studies indicated mutanases could be used to prevent caries. However, before they can be considered for more general use, problems relating to method of delivery, the short duration of contact with plaque, the slow diffusion of enzymes into plaque and the requirement for regular use need to be overcome.

Strain replacement therapy

There are two types of strain replacement therapy. The first involves pre-emptive colonization and the second relies upon competitive displacement.⁶⁴ In pre-emptive colonization studies, MS that were unable to produce caries, due either to their inability to produce lactic acid (lactate dehydrogenase mutants) or to synthesize intracellular polysaccharides (ICP mutants) were implanted into the oral microflora of experimental animals prior to introduction of potentially pathogenic MS.⁶⁴ The concept is that the non-virulent MS will have an ecological niche similar to that of virulent MS and thus will be capable of interfering with colonization by the cariogenic bacteria. The time in an infant's life when MS first colonize the teeth is the ideal period in which to

implant effector strains.²⁷ In competitive displacement, a non-cariogenic organism is introduced that is capable of competing with and displacing the indigenous cariogenic MS. An example of such a strain is *S.salivarius* strain TOVE-R (a rough colony variant of strain TOVE-S) which preferentially colonizes the tooth surfaces rather than the tongue.⁶⁵ Strain TOVE-R was shown capable of growing faster than MS⁶⁶ and, when given orally to rats, it soon became prominent in the animals' dental plaque and brought about a reduction in the levels of MS and dental caries.⁶⁵ The ideal effector strain would be a non-cariogenic bacterium which is continuously present in the mouth and which competes successfully with MS. The effector bacterium should accumulate preferentially on the tooth surfaces, be able to grow rapidly and withstand sudden and wide changes of pH.

Bacteriocin production may give effector strains an added advantage. Hillman et al used the bacteriocin-producing *S.mutans* strains JH1001 and JH1005 (the latter producing twice as much bacteriocin as the former) and the non-bacteriocin-producing strain JH1010 to colonize rats. These animals were then challenged with *S.mutans* strain Ingbritt. The minimum infective dose of strain Ingbritt was higher in animals that had been pre-emptively colonized with strain JH1005 and indeed this strain was more effective at excluding strain Ingbritt than was strain JH1001.⁶⁷ In another study, strain JH1001 was implanted on to the teeth of five human volunteers harbouring high levels of MS. Two and a half years later this strain was found to be still present in three subjects and in one of these no indigenous MS could be detected.⁶⁸ Despite the obvious population change brought about by the implantation of the colonizing strain, the total numbers of MS (colonizing strain plus indigenous MS) and of *S.sanguis* were not affected. Also, the persisting indigenous MS had not developed resistance to the bacteriocin produced by the implanted strain.⁶⁸ In another recent study, Hillman et al developed a LDH mutant (*S.mutans* BCS3-L1) and used this strain to colonize rats.⁶⁹ After 25 weeks, no revertants were observed and the caries scores of gnotobiotic and conventional rats were 61 and 48 per cent less respectively than the scores of comparable animals colonized with the parent strain, *S.mutans* JH1140. Caries scores obtained for BCS3-L1-colonized conventional rats were similar to those of *S.mutans*-free conventional animals. Also in this study, a spontaneous mutant of strain BCS3-L1 (producing three times as much mutacin as the parent strain) was isolated and this strain was considered to have 'supercolonization' potential with respect to the parent strain.⁶⁹

Strain replacement therapy potentially has many advantages. Long-term protection might theoretically be achieved following a single application of the effector strain, implying minimal cost. Furthermore, such strains may be naturally disseminated by interperson spread ('herd protection').⁷⁰

Eliminating established MS populations from the oral cavity

Mechanical removal of plaque

Studies by Axelsson et al showed caries can be prevented by regular toothbrushing and flossing.⁷¹ However, most studies have shown it is difficult to eliminate MS from pits, fissures and approximal surfaces by mechanical means alone. For effective caries control, these methods should be combined with the use of fluoride or other chemoprophylactic agents.

Chemoprophylactic agents

Ideally, chemoprophylactic agents should not be toxic or allergenic, stain the teeth, be absorbed through the oral or gastrointestinal mucosa, disturb the balance of the normal oral flora permitting opportunistic infections to develop or readily lead to resistance development.⁷² Chemoprophylactic agents considered for application to MS elimination include classical antibiotics; cationic agents such as chlorhexidine and cetylpyridinium chloride; plant-derived compounds such as sanguinaria extract; metal ions such as Zn²⁺ and Cu²⁺; anionic agents such as sodium dodecyl sulphate; and non-ionic agents such as triclosan.⁷³ These agents are generally delivered as mouthwashes or toothpastes but can also be applied in the form of gels or varnishes. The binding of these agents to oral surfaces and their subsequent slow release (substantivity) prolong their inhibitory effect.

Chlorhexidine and other chemical agents. Chlorhexidine, a bisbiguanide, has bacteriocidal activity against both gram-positive and gram-negative bacteria. Its effect against MS is greater than against *S.sanguis* and lactobacilli. Chlorhexidine treatment has been shown to reduce MS levels for periods of four-six months.⁷⁴ The reappearance of MS beyond six months has been attributed to the poor penetrating ability of chlorhexidine into plaque associated with pits, fissures and approximal surfaces. Because chlorhexidine is positively charged, it binds to various surfaces including enamel pellicle, hydroxyapatite and mucous membranes. It also binds to the negatively charged bacterial surface and disrupts bacterial cytoplasmic membranes, inducing leakage of low molecular weight components and the precipitation of cell contents. Chlorhexidine also inhibits key metabolic enzymes such as glucosyltransferase and phosphoenolpyruvate

phosphotransferase⁷³ and when combined with fluoride or metal ions such as Zn²⁺ has increased anti-cariogenic activity. This action is bacteriocidal, but when chlorhexidine elutes from oral surfaces at low levels its action may be bacteriostatic. A major part of the effectiveness of chlorhexidine is due to its substantivity; however, side-effects of its use include discoloration of teeth and taste disturbances.

Cetylpyridinium chloride is a quaternary ammonium compound with similar antimicrobial activity to chlorhexidine. However, following its adsorption to oral surfaces, it releases much faster than chlorhexidine and consequently has less sustained antibacterial activity than chlorhexidine.⁷⁵ Sanguinaria extract is a herbal preparation obtained from *Sanguinaria canadensis* by alcohol extraction.⁷³ The inhibitory effect of this preparation is inferior to that of chlorhexidine since it binds so strongly to surfaces that it has relatively poor bio-availability.⁷⁵ The bactericidal activity of the sanguinaria extract is due to interference with bacterial cell wall synthesis.⁷⁶ Sodium dodecyl sulphate (SDS) is a detergent commonly used in toothpastes. It has antimicrobial activity against a variety of bacteria including MS. The inhibitory effect of SDS against plaque growth is mainly due to its antimicrobial effect; however, SDS also competes with negatively charged bacteria and pellicle-binding proteins for binding sites, thus interfering with the early stages of plaque formation. In low concentrations, SDS may inhibit the glucosyltransferase activity of MS.⁷³ Triclosan is a phenolic compound with broad spectrum antimicrobial activity and reasonable substantivity.⁷⁷ The antibacterial action of this agent is due to interference with cell membrane function.⁷³ Strong *in vitro* activity was found when triclosan was combined with pyrophosphate.⁷⁷ Listerine is yet another phenolic product widely used as a mouthwash. The bactericidal effect of listerine is less than that of chlorhexidine.⁷⁸ Side-effects of its use include an unpleasant taste and burning sensation.

Classical antibiotics. Several researchers have explored the possible use of classical antibiotics to prevent dental caries. Pioneers in this field were McClure and Hewitt, who showed that caries incidence and *Lactobacillus acidophilus* counts in rats decreased following the administration of food or drinking water supplemented with penicillin.⁷⁹ Zander and Bibby subsequently reported that five of seven Golden Syrian hamsters whose teeth were brushed with penicillin-containing toothpastes were completely free of caries.⁸⁰ Fitzgerald observed a significant reduction of dental caries in Sprague-Dawley rats given 1-ephedrine penicillin intermittently with their food; a greater level of protection was observed when the antibiotic was given continuously. Also in this study, a reduction of

caries was observed when the rats received a wide variety of other antibiotics.⁸¹

In human studies, Løe et al observed that tetracycline, vancomycin and polymyxin mouthwashes reduced the formation of gingival plaque.⁸² Among these antibiotics, the greatest reduction was observed with tetracycline mouthwashes. The administration of vancomycin resulted in inhibition of gram-positive bacteria and caused a shift toward a gram-negative plaque flora. By contrast, polymyxin inhibited the gram-negative bacteria and caused a shift toward a gram-positive plaque flora.⁸² Lobene et al reported a four times daily administration of 250mg erythromycin as a liquid suspension (swallowed after three minutes rinsing) for seven days decreased the formation of plaque by 35 per cent but did not significantly alter the proportion of streptococci or extracellular polysaccharide-forming micro-organisms.⁸³ Jordan and De Paola reported a 10-minute daily application of 3 per cent vancomycin gel significantly decreased the numbers of *S.mutans* on sound teeth and in fissures of carious lesions after one week of treatment. A corresponding reduction in their numbers on smooth or proximal surfaces was not observed.⁸⁴ Loesche et al reported the application of kanamycin gel twice daily for one week reduced the number of *S.mutans* and *S.sanguis* immediately after gel treatment and a 46 per cent reduction of new caries was recorded 14-37 months following application of the gel.⁸⁵ Maltz and Zickert observed that 1-3.2g/day of penicillin V (taken orally as tablets) for 10 days decreased the numbers of *S.mutans* from 2.4×10^6 to 6.8×10^3 /ml saliva. No reduction was observed in the numbers of *S.sanguis* or lactobacilli. However, two days after the last administration of antibiotics, the *S.mutans* counts were almost the same as before treatment.⁸⁶

Most of the above studies demonstrated that classical antibiotics can prevent dental caries but, in general, these antibiotics cause imbalances within the normal flora, may cause resistance development in target organisms and may also predispose to opportunistic infections. Since dental caries is not a life-threatening disease, it is recommended that therapeutically applicable antibiotics should not be used for caries prevention.

Bacteriocin-like inhibitory substances. The term bacteriocin was initially used to describe the antibacterial proteins (colicins) produced by some strains of *Escherichia coli* that inhibit the growth of other *E.coli*.⁸⁷ Many studies have subsequently shown that bacteriocins are produced by a wide variety of both gram-positive and gram-negative bacteria. The term bacteriocin-like inhibitory substances (BLIS) was introduced to describe a variety of incompletely characterized proteinaceous inhibitors produced by gram-positive bacteria.⁸⁸

BLIS can be defined as bacterial peptide or protein molecules, released extracellularly, that in low concentrations are able to kill closely related bacteria by a mechanism against which the producer cell exhibits a degree of specific immunity. BLIS may inhibit sensitive bacteria by interfering with their metabolic activity, replication or viability⁸⁷ and BLIS having activity directed against MS (anti-mutans BLIS) could potentially be used for the prevention of dental caries.⁸⁹ Antimutans BLIS are most commonly produced by MS but may also be produced by other gram-positive bacteria.⁸⁹ There are several potential advantages in the use of bacteriocins or BLIS as anticaries agents, as they appear to be non-toxic and do not have any colour or taste.⁸⁷ However, MS may develop resistance to these agents.

Ikeda et al found a 59 per cent reduction in dental caries was achieved when specific pathogen-free rats infected with *S. mutans* were fed a diet containing bacteriocin C3603 (from *S. mutans* strain C3603). Also, application of this bacteriocin to bovine enamel slabs inhibited caries development in these slabs, both *in vitro* and when they were worn as oral appliances by human volunteers.⁹⁰ Fukushima et al found oral rinsing with bacteriocin RM10 (from *S. mutans* RM10) reduced the viable count of salivary bacteria in humans.⁹¹ Tsukamoto et al demonstrated that bacteriocin RM10 had the ability to induce chemotactic activity in polymorphonuclear leucocytes and monocytes.⁹² So, in addition to its bactericidal effect, this bacteriocin appeared to also enhance the natural antibacterial defences of the host.⁹² Loyola-Rodriguez et al showed that bacteriocin 6223 (from *S. sobrinus* strain 6223), when incorporated into the drinking water of specific pathogen-free rats, prevented the development of caries in animals fed a sucrose-containing diet and challenged with *S. mutans* strain MT8148⁹³

Increasing the acid-resistance of the teeth

Fluoride and phosphates

Increased tooth resistance to caries development may be achieved by the use of fluorides; indeed the use of fluoride in toothpaste and other oral products is believed to be the major reason for the substantial decline in caries incidence in many developed countries.¹ Fluorides can be administered systemically (tablets), applied topically (toothpastes or mouthwashes) or applied by dentists in the form of solutions, gels and varnishes. In some parts of the world, fluoride is added to drinking water. Fluoride binds to hydroxyapatite crystals to form fluoroapatite, which is relatively resistant to demineralization and also stimulates remineralization of the tooth.⁹⁴ In addition to these chemical effects, fluoride directly inhibits the MS enzyme enolase, leading to reduced

glucose uptake through the phosphotransferase system and ultimately less glycolysis and ICP synthesis. If fluoride is given excessively, it may cause fluorosis, a condition in which brown discoloration and mottling of the teeth occurs. A number of studies have shown that continuous exposure to fluoride leads to development of fluoride-resistant MS.⁹⁵ However, these fluoride-resistant bacteria are non-cariogenic because they do not produce sufficient acid to cause demineralization of the enamel.

Phosphates have also been used as food additives to prevent dental caries. It was reported that the addition of sodium trimetaphosphates to chewing-gum⁹⁶ and calcium sucrose phosphate to the diet prevented dental caries.⁹⁷ In general, inorganic phosphates promote remineralization and, when present in high concentrations, decrease acid production by preventing the activation of lactate dehydrogenase by fructose diphosphate.⁹⁸

Pit and fissure sealants

Treating primary and permanent molars with pit and fissure sealants prevents colonization by MS and precludes penetration of the fissure by acid. Furthermore, any bacteria present in the pits and fissures prior to application of the sealant will become quiescent due to restriction of their access to nutrients.⁹⁹

Control of the carbohydrate composition of the diet

It has been demonstrated that restriction of dietary sucrose reduces the level of MS. Sucrose is important for both glucan-mediated adhesion and acid production. Xylitol, sorbitol, saccharin and aspartame have all been used as sugar substitutes for the purpose of reducing dental caries in a wide variety of products including sweets, candies, chewing-gum, oral hygiene products and pharmaceutical products. Xylitol is a five-carbon sugar that has the same sweetness as sucrose, but is not fermented by MS. Xylitol is transported across the cell membrane by a phospho-transferase system, generating xylitol-5-phosphate. Xylitol-5-phosphate may subsequently be dephosphorylated and exported from the cell, thus creating a futile cycle that consumes cellular ATP.¹⁰⁰ In addition, the xylitol-5-phosphate and its metabolite xylulose-5-phosphate have been shown to inhibit phosphofructokinase, thus causing depletion of the energy-generating potential of the cell.¹⁰⁰ Xylitol also promotes remineralization of the tooth, so early carious lesions can be arrested.¹⁰¹ Sorbitol has also been used as a sugar substitute, sometimes in combination with xylitol. This six-carbon sugar is cheaper than xylitol but its sweetness is only 50 per cent that of sucrose or xylitol.

Although sorbitol can be fermented by MS, the rate of acid production is significantly lower than from other dietary sugars such as sucrose, glucose and fructose.¹⁰²

Saccharin has been used for many years as a sugar substitute. It has a structure similar to sulfonamide,¹⁰³ is 300 times sweeter than sucrose, but it has a bitter taste when used at concentrations greater than 0.1 per cent. It was reported that, in high concentrations (0.5 per cent), saccharin can prevent dental caries,¹⁰⁴ but physiological concentrations (33.3mg per cent) failed to have any effect.¹⁰⁵ Saccharin inhibits the growth of MS due to competitive inhibition of lactate dehydrogenase.⁹⁴ Aspartame, a dipeptide comprising aspartic acid and phenylalanine,¹⁰³ is 200 times sweeter than sucrose and is used extensively in sugar-free soft drinks and chewing-gum. It appears to inhibit MS metabolism.⁹⁴

Conclusion

It is now known that MS are the principal aetiological agents of dental caries. This group of streptococci comprises seven species, of which *S. mutans* and *S. sobrinus* are most important in terms of human caries. MS generally colonize the teeth soon after they erupt and the principal source of infection is the child's mother. Cariogenic features of these bacteria include synthesis of water-insoluble glucans, lactic acid production, ability to survive at a low pH, intracellular polysaccharide synthesis and the production of a dextran-hydrolyzing enzyme (endodextranase).

Potentially, caries can be reduced by interfering with transmission of MS, eliminating established MS populations from the oral cavity, increasing the acid-resistance of teeth and control of the carbohydrate composition of the diet. Although apparently safe and efficacious oral anti-mutans vaccines have been demonstrated in the laboratory, the costs involved in their development for human use are relatively high. Application of glucan-hydrolyzing enzymes to prevent the attachment of MS to hard surfaces has been tried, with little success. Animal studies suggest there is great promise in the implantation of benign oral microbial strains capable of successfully competing with MS, but few human trials have been undertaken. Mechanical methods of plaque control including brushing, flossing and professional scaling are only temporarily effective in eliminating MS. The control of plaque growth by chemical means has attracted considerable attention and chlorhexidine has been shown to be effective, but causes discoloration of the teeth with prolonged use. Classical antibiotics can certainly interfere with plaque development, but they are not appropriate for long-term application because of their medical significance, and because they can lead to resistance

development and ecological imbalances favouring opportunistic infections. The relatively narrow-spectrum natural bacterial antibiotics known as bacteriocins (or BLIS) appear to offer considerable potential benefits as anti-MS agents. These small peptide molecules could be incorporated into mouthwashes or toothpastes or, alternatively, antimutans BLIS-producing bacteria could be implanted within the oral microbiota. Fluoride is still the best available anti-caries chemical agent; its anti-caries action is attributable to increasing the resistance of the tooth to acid demineralization, stimulation of remineralization and inhibition of MS carbohydrate metabolism. Although sugar substitutes could potentially play an important role in caries control, consumer preference continues to overwhelmingly favour the use of sucrose.

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Address for correspondence/reprints:
Associate Professor John R Tagg
Department of Microbiology
University of Otago
PO Box 56
Dunedin, New Zealand