Combating dental decay

Stuart Dashper and Eric Reynolds

Section of Oral Biology
School of Dental Science
The University of Melbourne
711 Elizabeth St
Melbourne VIC 3000
Tel: (03) 9341-0434
Fax: (03) 9341-0236
E-mail:
stuartgd@unimelb.edu.au

Dental caries

Dental caries or tooth decay is one of the most prevalent bacterial infectious diseases of mankind. In recent oral health surveys, more than 60% of Australian teenagers surveyed had experienced the disease and most dentate adults surveyed exhibited multiple teeth affected by caries. Treating the consequences of dental caries accounts for over 50% of the total cost of providing dental services in Australia, which in 1998 was estimated at $2.6 billion [1]. Dental caries is a dynamic process that is initiated by microbial biofilms on the tooth surfaces (dental plaque) resulting in a disturbance of the equilibrium between tooth mineral and the surrounding plaque fluid so that over time there is a net loss of mineral from the tooth surface. This demineralisation of the enamel may ultimately lead to cavitation of the surface of the tooth and once this stage of the disease has been reached only restorative methods (fillings) can be employed to limit the spread of decay and eventual loss of the tooth. Whilst many of the over 700 species of oral bacteria are saccharolytic and ferment dietary sugars to acidic end products few are able to produce acid at a sufficient rate to overcome salivary buffering capacity and rapidly lower plaque pH. Most species are also unable to continue metabolism in the acidic environment (approximately pH 5.2) that is necessary to cause enamel demineralisation and initiation of the caries process. The main bacterial species associated with caries initiation are members of the 'mutans streptococcus group' as these species are both strongly acidogenic and aciduric [2,3,4]. Along with animal model testing, analysis of many clinical studies has indicated that Streptococcus mutans, Streptococcus sobrinus and Lactobacillus casei are the species most commonly associated with dental caries [5]. These species are part of the normal oral microflora and are regarded as opportunistic pathogens. The application of new technologies such as transcriptomic and proteomic analyses will provide more detailed insights into the virulence factors of these species [6,7]. Fluoride has been the main weapon in the arsenal against caries and is undoubtedly one of the most effective preventive therapies. However, its extensive use has failed to eliminate the disease and recent Australian and overseas survey data indicate that caries prevalence in the community is no longer decreasing and in some groups is increasing [8].

Novel preventive and treatment strategies

Much of the microbiological research into caries prevention is based on the belief that *S. mutans* is the major causative agent of caries initiation and development and that removal of this bacterium from dental plaque or modification of its virulence will stop or greatly reduce the incidence of dental caries in individuals – a still as yet untested hypothesis.

Replacement therapy

Jeff Hillman and colleagues at the University of Florida, Gainesville have developed a novel 'replacement therapy' where the indigenous strains of *S. mutans* are displaced by an avirulent strain. Convinced that the major virulence factor of *S. mutans* is its ability to produce lactic acid at a rapid rate, these researchers have genetically engineered a strain of *S. mutans* to produce ethanol instead of lactic acid. This was achieved by replacing the *S. mutans* lactate dehydrogenase gene with a *Zymomonas mobilis* ethanol dehydrogenase gene. In batch culture, the mutant produced less acid than the wild-type strain. However, its slower growth rate did not give it the competitive edge needed to displace the indigenous strains of *S. mutans* already present in the mouth. This competitive edge was provided by inserting a naturally occurring plasmid into the already modified ethanol-producing strain. The plasmid encoded a 'lantibiotic' (mutacin 1140) that is lethal to most other *S. mutans* strains [9]. Animal testing indicated that this strain did displace other *S. mutans* strains and lead to a reduced level of caries, but concerns about the introduction of genetically modified organisms to the oral cavity have so far slowed clinical trials in humans. The technology is currently being pursued by Oragenics (http://www.oragenics.com) who have been refused permission to test this replacement therapy in Phase I human trials because of fears of horizontal transmission of the genetically modified bacterium. Recently, permission has been granted to undertake a Phase I safety trial using an auxotrophic strain and denture wearing participants to determine the level of

transmission of the bacterium. Given the level of caution about testing a genetically modified bacterium it begs the question, will consumers be willing to allow a mouth rinse containing genetically modified bacteria to be administered to their children to slow the development of caries, even if the technology is shown to be effective?

Caries vaccine

The development of caries vaccines has been investigated since the late 1960s but has met with little success due to the location of the bacteria in fissures of the back teeth that are inaccessible to antibodies and the potential cross-reactivity of streptococcal antigens. The best results to date have been obtained by stimulating a salivary IgA response against *S. mutans,* which only resulted in a short term suppression of the bacterium in plaque [10]. However, proponents of this strategy contend that improvements in vaccine delivery technology and the characterisation of species specific surface proteins will improve results.

Julian Ma, Tom Lehner and colleagues from Guy's Hospital, London have developed a novel, passive vaccine against *S. mutans.* In an impressive piece of research, they purified the major *S. mutans* adhesin (SpaI/II) and used this to produce a monoclonal antibody from murine spleen cells. The genes encoding the monoclonal antibody were then inserted into a tobacco plant engineered to over-express the assembled antibody [11]. This recombinant monoclonal antibody, or plantibody, binds specifically to *S. mutans* and interferes with its ability to colonise dental plaque. Prior to antibody application, antiseptic treatment is used to greatly reduce the levels of *S. mutans* and many other bacteria in plaque. The antibody interferes with adhesion of *S. mutans* to plaque whilst recolonisation by other oral bacterial species occurs unimpeded. This treatment is reported to ecologically remove *S. mutans* from the mouth for up to six months. Animal testing of the monoclonal antibody demonstrated a sustained reduction in *S. mutans* in plaque and a corresponding decrease in caries. This technology is now being commercialised by the Planet company as CaroRx™. This treatment has completed US FDA Phase I clinical trials and is currently undergoing Phase II clinical trials.

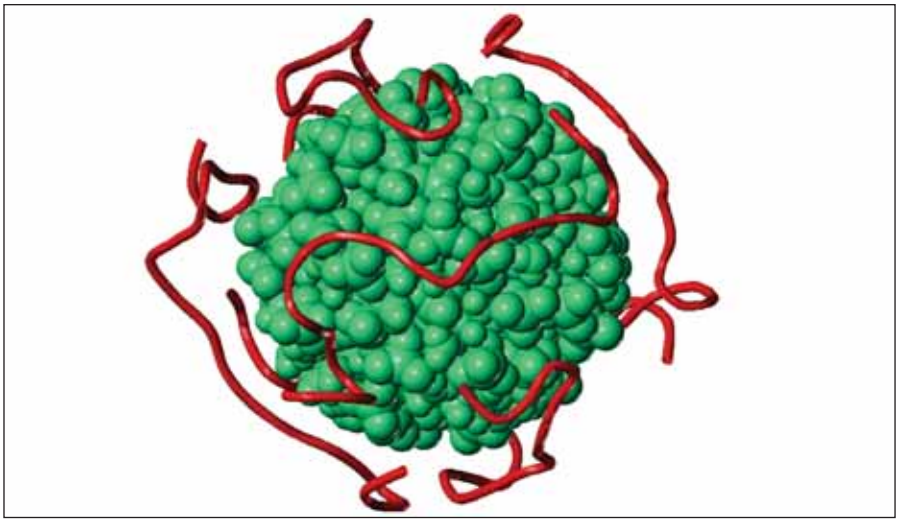
Anticariogenic peptides

At the CRC for Oral Health Science, we have taken a more biochemically-based approach to caries prevention by developing a technology that increases the concentration of calcium and phosphate, the major components of enamel, at the tooth surface. Using synthetic phosphopeptides we identified the motifs (-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-) within the bovine milk protein casein, responsible for stabilisation of amorphous calcium phosphate allowing milk to become supersaturated with bioavailable calcium and phosphate [12]. By extracting these phosphopeptides we have produced a novel, bioavailable, amorphous form of calcium phosphate (ACP) stabilised by the casein phosphopeptides (CPP). The stabilised CPP-ACP form nanocomplexes and their 3D structure demonstrate the way in which the casein phosphopeptides act to stabilise a core of calcium and phosphate that maintains supersaturation (Figure 1) [13]. The CPP-ACP nanocomplexes substantially increase the levels of calcium and phosphate in dental plaque thereby depressing enamel demineralisation and facilitating remineralisation. Extensive testing in animal and *in situ* trials has demonstrated the ACP-CPP nanocomplexes to be anticariogenic. This technology has been patented and developed commercially and is available world-wide as the anticariogenic additive Recaldent™.

Antibacterial peptides

Continuing with the theme of investigating bioactive peptides derived from bovine milk we have discovered a novel antimicrobial peptide, Kappacin that has activity against *S. mutans* growing as a biofilm [14,15]. *In vivo,* oral streptococci grow as part of a complex polymicrobial biofilm (supragingival dental plaque) adhering to the surface of the tooth, embedded in a matrix of bacterial and host polymers. Growth as a biofilm offers a number of significant advantages to the bacterium over planktonic growth not the least of which

Figure 1. Molecular model of a casein phosphopeptide amorphous calcium phosphate nanocluster. The red ribbons depict the casein phophopeptides bound to the surface and stabilising amorphous calcium phosphate which is depicted in green.



is protection against antimicrobial agents. We have developed methods for the culture of *S. mutans* and other oral bacteria as biofilms in a constant depth film fermenter (CDFF). This device provides a sophisticated means of reproducing large numbers of biofilms that can be used for antimicrobial testing. Unlike testing against planktonic bacteria, results obtained with the CDFF have a predictive value on the efficacy of the antimicrobial agent in the oral cavity. An antimicrobial peptide that is able to decrease *S. mutans* levels in plaque has potential in combating caries.

New opportunities

The growth of oral streptococci as part of a polymicrobial biofilm also offers the opportunity to interfere with the intercellular signalling systems that many of these species use to communicate and regulate gene expression. Increasingly, the quorum-sensing peptides of these bacteria look to hold promise for manipulating the composition of oral biofilms and, possibly, modulating their virulence [16].

Conclusion

The only effective anticaries agent that has been developed in the last fifty years is fluoride. However, recent research has resulted in the development of other agents (specific antibodies, CPP-ACP and antibacterial peptides) that show promise in supplementing the action of fluoride to help prevent dental caries.

References

1. Armfield J, Roberts-Thomson K and Spencer A. 2000 Australia's Health 2000: the seventh biennial health report of the Australian Institute of Health and Welfare. Canberra.
2. Marsh 2003 Are dental diseases a result of ecological catastrophes? *Microbiology* 149: 279-294.
3. Dashper, SG and Reynolds, EC. (1992) pH Regulation in *Streptococcus mutans*. *J Dent Res* 71:1159-1165.
4. Dashper, SG and Reynolds, EC. (2000) Effects of organic acids on growth, glycolysis and intracellular pH of oral streptococci. *J Dent Res* 79:90-96.
5. Loesche WJ. (1986) Role of *Streptococcus mutans in human dental decay. Microbiol Rev* 50:353-380.
6. Len A, Harty DWS and Jacques NA. (2004) Proteome analysis of *Streptococcus mutans* metabolic phenotype during acid tolerance. *Microbiology* 150:1353-1366.
7. Svensater G, Welin J, Wilkins J, Beighton D and Hamilton I. (2001) Protein expression by planktonic and biofilm cells of *Streptococcus mutans*. *FEMS Microbiol. Lett.* 205:139-146.
8. Spencer AJ. (2004) Narrowing the inequality gap in oral health and dental care in Australia. Australian Health Policy Institute. Commissioned Paper Series.
9. Hillman JD, Brooks TA, Michalek SM, Harmon CC, Snoep JL and Van Der Weijden CC. (2000) Construction and characterization of an effector strain of *Streptococcus mutans* for replacement therapy of dental caries. Infect Immun 68:543-549.
10. Hajishengallis G and Michalek SM. (1999) Current status of a mucosal vaccine against dental caries. *Oral Microbiol Immunol* 14:1.
11. Ma J, Hikmat B, Wycoff K, Vine N, Chargelegue D, Yu L, Hei, M and Lehner T. (1998) Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. *Nature Medicine* 4:601-606.
12. Reynolds EC. (1999) Anticariogenic casein phosphopeptides. *Prot Peptide Lett.* 6:295-303.
13. Cross KJ, Huq NL, Palamara JE, Perich JW and Reynolds EC. (2005) Physicochemical characterization of casein phosphopeptide-amorphous calcium phosphate nanocomplexes. *J Biol Chem.* 280:15362-15369.
14. Dashper SG, O'Brien-Simpson, NM, Cross K, Paolini, RA, Hoffman, B, Catmull D, Malkoski, M, and Reynolds EC. (2005) Divalent metal cations increase the activity of the antimicrobial peptide Kappacin. *Antimicrob Agents Chemother* In Press 2005.
15. Malkoski M, Dashper SG, O'Brien-Simpson NM, Talbo GH, Macris M, Cross KJ and Reynolds EC. (2001) Kappacin, a novel antibacterial peptide from bovine milk. *Antimicrob Agents Chemother* 45:2309-2315.
16. Cvitkovitch D, Li Y-H, and Ellen R. (2003) Quorum sensing and biofilm formation in streptococcal infections. *J Clin Invest* 112:1626–1632.