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An *in Vitro* and *in Vivo* Investigation of the Anti-caries Activity of Medium-chain Fatty Acids

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Fatty acids (FA) have been known to have a caries-protective effect for many years and show bactericidal and surface-active properties. This work was undertaken to compare the degree of caries protection offered by medium-chain FA with chain lengths of 9~12 carbons and to assess the anti-caries potential of sodium laurate, which showed the greatest anti-caries effect among the FA tested. The sodium salts of FA significantly reduced plaque bacterial metabolism by 7 standard strains of acidogenic bacteria in a dose-dependent manner and the order of bacterial metabolism reduction was from sodium laurate to decanoate to nonanoate. Because sodium laurate showed maximum anti-caries potential *in vitro*, we further examined directly its anti-caries effect using an *in vivo* rat model. Sodium laurate also produced a highly significant reduction in caries occurrence compared to that of control and gave a greater degree of caries prevention than fluoride *in vivo*. Our results indicate that the salt form of laurate has a very strong anti-caries effect *in vitro* and *in vivo*.

Keywords: medium-chain fatty acids, acidogenic plaque bacteria, acid production, polysaccharide synthesis, rat caries.

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Introduction

Fatty acids (FA), which are plentiful in human foods, such as oils, fats, and milk, have been known to have caries-protective effects for many years (Nikiforuk, 1970; Hayes, 1984). Moreover, FA and their esters are recognized antimicrobial agents, showing bactericidal, surface-active properties because they act as anionic and non-ionic surfactants, respectively (Lynch *et al.*, 1983), and this causes their anti-caries effect. Among the FA, medium-chain FA with a chain length of 9~12 possess the optimal balance between water and lipid solubilities at pH 5.5, and maximally inhibit plaque bacterial acid production when included in the soluble salt form in a carbohydrate-rich medium (Cole and Eastoe, 1988; Hayes, 1994). If and when carbohydrate is metabolized to acids, they are converted into their lipid-soluble protonated form, which dissociates on entering the cell, and thus this causes a shuttling of protons across the membrane, and cytoplasmic acidification in the organism by disrupting the transmembrane pH gradient (Hayes and Roden, 1990). Therefore, pellicle lipids may well not only modulate the invasion of the tooth surfaces by bacteria, but also control the extent of enamel demineralization by acids (Slomiany *et al.*, 1986).

Similarly, fluoride ions are also taken into the cell in a lipid-soluble protonated form when the external pH is lower than the internal pH, and this too leads to internal acidification. In so doing, the fluoride ions inhibit glycolytic enzymes, such as phosphofructokinase, which are inactivated by a reduction of the intracellular pH, and glycolytic enzyme enolase (Cole and Eastoe, 1988). In addition, fluoride acts by inhibiting

the proton-translocating membrane ATPase (Sutton and Marquis, 1987). More importantly, fluoride and medium-chain FA have been used practically as anti-caries agents, because they were found to reduce sugar-induced dental caries in experimental animals when included in the diet (Hayes and Stobart, 1990).

In this study, we examined the degree of caries protection by medium-chain FA with chain lengths of 9~12 carbons and assessed the anti-caries potential of sodium laurate, which showed the greatest anti-caries effect among the FA tested. To evaluate the anti-caries potential of medium-chain FA tested *in vitro*, 7 standard strains of acidogenic bacteria were examined in this study. Direct caries prevention was further evaluated using an *in vivo* rat model.

Materials and Methods

Microorganisms and chemicals

The bacterial species investigated, *S. mutans* 10449, *S. mutans* JC-2, *S. mutans* BHT, *S. sanguis* 10556, *L. casei* ATCC 4646, *L. casei* ATCC 27216, and *A. viscosus* ATCC 15987, were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The sodium salts of nonanoic, decanoic, and lauric acids were obtained from the Sigma (St. Louis, MO, USA).

Determination of acid production (fall in pH), bacterial growth, and extracellular polysaccharide formation

Aliquots (0.1 ml; OD_{600}, 0.5) of a bacterial suspension were added to 5.9 ml of an appropriate medium (streptococci, Todd-Hewitt broth; lactobacilli, Lactobacilli MRS broth; actinomyces, Brain Heart Infusion broth) containing 2% glucose and 0.5, 1, 2, 4, or 8 mM of sodium nonanoate, sodium decanoate, or sodium laurate, which was adjusted to a pH 7.0 or 5.0. The mixtures were incubated anaerobically at 37°C for 24 h (streptococcus and lactobacillus) or 48 h (actinomyces). After incubation, the cultures were clarified by centrifugation at 5,000× *g* for 15 min and the supernatant was then processed to determine final pH values using a digital pH meter. To determine the effect of medium-chain FA on bacterial growth, optical density of the cultures was measured spectrophotometrically at 600 nm. Cultures incubated in the presence of the FA without bacterial cells and cultures incubated in the absence of the FA with bacterial cells served as negative and positive controls, respectively.

To determine the effect of medium-chain FA on extracellular polysaccharide synthesis, 5.9 ml of a medium (streptococci, Todd-Hewitt broth; lactobacilli, Lactobacilli MRS broth; actinomyces, Brain Heart Infusion broth) supplemented with 2% glucose and 1 mM of sodium nonanoate, sodium decanoate, or sodium laurate, which was adjusted final pH to 7.0, was inoculated with 0.1 ml of a bacterial suspension (OD_{600}, 0.5). The mixtures were then incubated anaerobically at 37°C for 24 h (streptococcus and lactobacillus) or 48 h (actinomyces). After incubation, the cultures were pelleted by centrifugation at 5,000 × *g* for 15 min. To extract soluble extracellular polysaccharides, 3 times the volume of methanol was added to supernatant. Soluble extracellular polysaccharides were precipitated by centrifugation at 5,000 × *g* for 10 min and the precipitate was dissolved in distilled water; the procedure was then repeated. To extract the insoluble extracellular polysaccharides, the pellet was washed with 0.22 M phosphate buffer (pH 7.4) twice and 0.5 N NaOH was added. After incubation for 90 min, it was centrifuged and supernatant was neutralized with 1 N HCl. Three times its volume of methanol was then added and the insoluble extracellular polysaccharides were precipitated by centrifugation. The amounts of water-soluble or water-insoluble extracellular polysaccharides were quantified by phenol-sulfuric acid assay (DuBois *et al.*, 1956). Cultures incubated in the presence of the FA without bacterial cells and cultures incubated in the absence of the FA with bacterial cells served as negative and positive controls, respectively.

Effect of sodium laurate on caries prevention in rats

To test the effect of sodium laurate on caries formation in a rat model, we determined the degree of caries formation after placing the animals on a cariogenic diet plus 50 mM sodium laurate by scoring rat molars by the Larson modification of Keye's method (Larson, 1981) under a light microscope. In brief, forty-five 4-week-old Sprague-Dawley rats, having an initial body weight of between 190 and 210 gm were used. Fifteen animals were assigned to each group, each group being balanced for mean initial body weight and gender and distinguished on the basis of their diets and drinking water. The cariogenic diet group was fed a mixture of foods containing 40% sucrose, 32% dry milk, 21% flour, 5% yeast, 2% anchovy, 0.01% vitamin B, and 0.01% vitamin C. The NaF-containing diet group was fed a cariogenic diet and drinking water containing 2.64 mM NaF (50 ppm F). Finally, the sodium laurate-containing diet group was fed a cariogenic diet containing 50 mM sodium laurate. Throughout the period of monitoring, the rats were given water ad libitum. After 120 days, all tested animals were sacrificed with ether and their maxillas and mandibles were removed. Specimens were fixed in 10% formalin and a caries index assigned, as previously described (Larson, 1981). In brief, food debris in fissures and pit of molars were removed with a barbed broach. Specimens containing the first, second, and third molars were cut out with Exakt cutting system (Exakt-Apparateb, Hamburg, Germany). Maxillary and mandibular molars were hemisectioned in a mesiodistal sagittal plane freehand with a cutting disk (0.004 inch in thickness, 0.75 inch in diameter). For staining, specimens were incubated with 0.02% ammonium prupurate solution and washed for 10 min with distilled water. They were then incubated with ammoniacal silver nitrate solution for 30 sec and washed for

2 min with distilled water. Depth of penetration of dental caries was determined using an Olympus BH-2 light microscope and recorded using the following classification: enamel only (E), slight in dentin (Ds), moderate in dentin (Dm), and extensive in dentin (Dx). Scores of linear units were added using the Larson modification of Keye's method (Larson, 1981) and the total score used as a caries index. Number of linear units assigned to each molar was presented in Table 1. Mann-Whitney U test was used to evaluate the statistical significances of differences between specific diet groups.

Statistical analysis

The results in acid production and bacterial growth after incubation in the presence of the FA were analyzed by Student's *t*-test. Statistical significance was defined as $P < 0.01$.

Table 1. Number of linear units assigned to each molar

Lesion types	Molars					
	Mandibular			Maxillary		
	1st	2nd	3rd	1st	2nd	3rd
Sulcal	7	5	2	5	3	2
Proximal	1	2*	1	1	2*	1

*One mesial and one distal unit.

Results

Medium-chain FA inhibit acid production by acidogenic plaque bacteria and decrease bacterial growth

To explore the effect of medium-chain FA on acid production (fall in pH) by acidogenic bacteria, extracellular pH values in cultures were measured for 7 standard strains of acidogenic plaque bacteria, after incubating them in an appropriate medium containing 2% glucose and 0.5 to 8 mM of medium-chain FA, adjusted to a pH of 7.0 or 5.0. To investigate whether acid production is associated with bacterial growth, we also measured optical density in cultures exposed by medium-chain FA in a similar condition. When the bacteria were exposed to 0.5 to 8 mM of sodium nonanoate, sodium decanoate, or sodium laurate, acid production by all strains of both streptococcus and lactobacillus tested were significantly inhibited at both pH 7.0 and 5.0 in a dose-dependent manner (Fig. 1). Sodium laurate showed the highest level of inhibition of acid production by acidogenic bacteria at both pH 7.0 and 5.0 (Figs. 1E, 1F), and sodium nonanoate showed the lowest level under the same condition (Figs. 1A, 1B). In particular, acid production by all strains of both streptococcus and lactobacillus was significantly inhibited at even low concentrations (1 mM) of sodium laurate. Such changes, however, were not observed from the strain tested (ATCC 15987) of *A. viscosus*, which was known as a weakly acidogenic bacterium among Gram-positive organisms but can cause root caries in animals (Cole

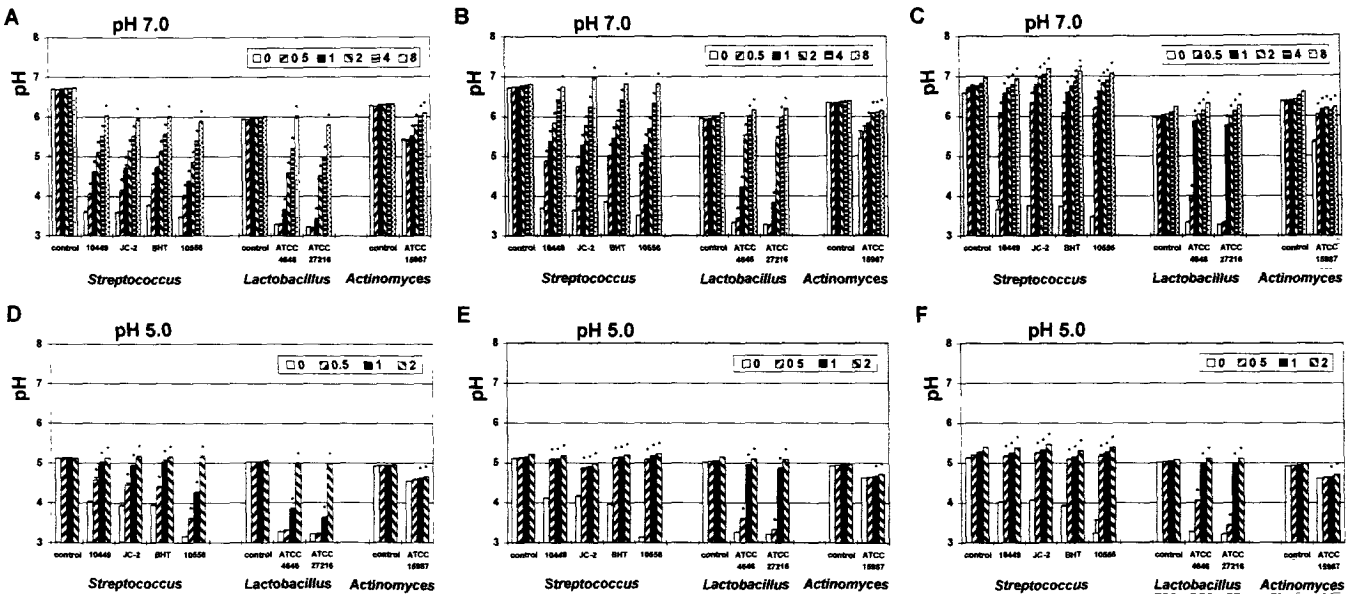

Fig. 1. Effects of sodium nonanoate (A, D), sodium decanoate (B, E), and sodium laurate (C, F) on acid production (fall in pH) by standard strains of *S. mutans*, *S. sanguis*, *L. casei*, and *A. viscosus*. Seven standard strains of acidogenic plaque bacteria were cultured anaerobically at 37°C for 24 h (streptococcus and lactobacillus), or 48 h (actinomyces) in a medium (streptococci, Todd-Hewitt broth; lactobacilli, Lactobacilli MRS broth, actinomyces, Brain Heart Infusion broth) containing 2% glucose and 0.5, 1, 2, 4, or 8 mM medium-chain FA, adjusted to either pH 7.0 or 5.0. After incubation, cultures were clarified by centrifugation and the supernatant was then processed to determine final pH values. Cultures incubated in the presence of the FA without bacterial cells and cultures incubated in the absence of the FA with bacterial cells served as negative and positive controls, respectively. Values represent mean ± SD from six independent determinations. *Significantly different from the positive control at $P < 0.01$.

and Eastoe, 1988), in a similar condition. These results indicate that the sodium salts of the medium-chain FA with chain lengths of 9~12 significantly inhibited acid production by acidogenic plaque bacteria under both neutral and acidic conditions in a dose-dependent manner. Moreover, the order of bacterial acid inhibition ability was from sodium laurate (highest) to sodium decanoate and sodium nonanoate. Our results also demonstrate that low concentration (1 mM) of sodium laurate significantly inhibited acid production by acidogenic plaque bacteria under acidic condition as well as at neutral pH. When the bacteria were exposed to 0.5 to 2 mM of sodium nonanoate, sodium decanoate, or sodium laurate, bacterial growth of all strains of streptococcus, lactobacillus, and actinomyces tested were significantly decreased at both pH 7.0 and 5.0 in a dose-dependent manner (Fig. 2). Sodium laurate showed the highest level of inhibition of bacterial growth at both pH 7.0 and 5.0 (Figs. 2C, 2F), and sodium nonanoate showed the lowest level under the same condition (Figs. 2A, 2D). In particular, bacterial growth in all strains of both streptococcus and lactobacillus was significantly inhibited at even low concentrations (1 mM) of sodium laurate. Such changes, however, were not observed from the strain tested (ATCC 15987) of *A. viscosus*, in a similar condition. These results indicate that the sodium salts of the medium-chain FA with chain lengths of 9~12 significantly inhibited bacterial growth under both neutral and acidic conditions in a dose-dependent manner. These results also suggest that the sodium salts of the medium-chain FA with chain lengths of 9~12 inhibit acid production by decrease of bacterial growth in acidogenic plaque bacteria.

Medium-chain FA inhibit polysaccharide synthesis by acidogenic plaque bacteria

As we described above, low concentrations (1 mM) of sodium laurate significantly inhibited acid production by standard strains of acidogenic plaque bacteria. Therefore, to study the inhibitory effect of 1 mM medium-chain FA upon extracellular polysaccharide synthesis by acidogenic bacteria, 7 standard strains of acidogenic plaque bacteria were cultured in a medium (pH 7.0) containing 2% glucose and 1 mM medium-chain FA and the amount of extracellular polysaccharides determined. Water-soluble forms comprised the major portion of the polysaccharides produced by all bacterial strains tested, and only a small quantity of the water-insoluble forms was produced. When the bacteria were exposed to 1 mM of sodium nonanoate, sodium decanoate, or sodium laurate, the amount of water-soluble polysaccharide produced was significantly reduced, compared to the appropriate controls (Table 2). In addition, sodium laurate inhibited soluble polysaccharide synthesis most in all bacterial strains tested at pH 7.0, and sodium nonanoate the least under the same condition. Though bacterial strains tested produced only a small amount of water-insoluble polysaccharides, this too was reduced by exposure to 1 mM medium-chain FA. These results indicate that the sodium

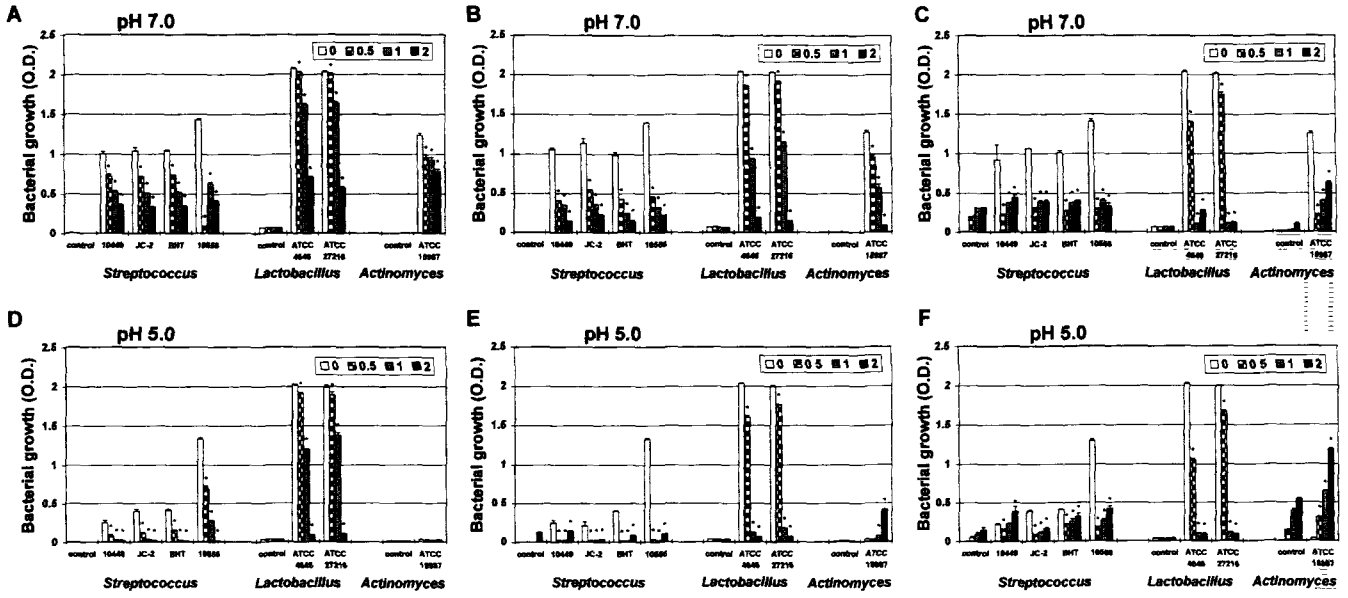

Fig. 2. Effects of sodium nonanoate (A, D), sodium decanoate (B, E), and sodium laurate (C, F) on bacterial growth by standard strains of *S. mutans*, *S. sanguis*, *L. casei*, and *A. viscosus*. Seven standard strains of acidogenic plaque bacteria were cultured anaerobically at 37°C for 24 h (streptococcus and lactobacillus), or 48 h (actinomyces) in a medium (streptococci, Todd-Hewitt broth; lactobacilli, Lactobacilli MRS broth; actinomyces, Brain Heart Infusion broth) containing 2% glucose and 0.5, 1, or 2 mM medium-chain FA, adjusted to either pH 7.0 or 5.0. After incubation, cultures were processed to determine optical density values. Cultures incubated in the presence of the FA without bacterial cells and cultures incubated in the absence of the FA with bacterial cells served as negative and positive controls, respectively. Values represent mean ± SD from six independent determinations. *Significantly different from the positive control at $P < 0.01$.

salts of medium-chain FA with a chain length of 9~12 significantly inhibited polysaccharide production by acidogenic plaque bacteria, in the order sodium laurate > sodium decanoate > sodium nonanoate.

Effect of sodium laurate on caries prevention in rats

To investigate the effect of sodium laurate on caries prevention in rats, we determined the linear units assigned to each molar using the Larson modification of Keye's method (Larson, 1981) after placing the rats on a cariogenic diet plus sodium laurate. All animals remained healthy and alert during the 120-day experimental periods, and consumed the same amounts of food and water though on different diets and the addition of sodium laurate to the diet had no effect on the mean weight gain. Table 3 showed the linear unit reductions in sulcal carious lesions obtained using sodium laurate- and fluoride-containing diets compared to that of the cariogenic diet group. In the case of sulcal caries lesions, the mean value of the linear units of the cariogenic diet group was highest and the sodium laurate-containing diet group lowest. Surprisingly, caries experience in the sulcal caries lesions of rats maintained on the sodium laurate-containing diet was lower than that of NaF-containing diet group. In addition, mandibular molars had a higher frequency of sulcal caries than maxillary molars. These results demon-strate that sodium laurate decreases caries formation in experimental animals. However, no variations in the fre-quencies of proximal caries was found among the different groups of rats maintained on the cariogenic, NaF-, and laurate-containing diets during the 120-day experimental periods (Table 4).

Discussion

This study shows that the sodium salts of medium-chain FA with a chain length of 9~12 significantly inhibited both bacterial sugar metabolism and plaque matrix formation. The production of both acids and extracellular polysaccharides by the FA was significantly and dose-dependently inhibited by increasing the carbon chain length in all strains of streptococcus and lactobacillus tested. In particular, a low concentration (1 mM) of sodium laurate significantly inhibited the production of both acids and polysaccharides by acidogenic bacteria under acidic (pH 5.0) and neutral conditions *in vitro*. These results are in agreement with a previous report, which showed that FA with chain lengths of 8~15 inhibit the production of both acids and polysaccharides in *S. mutans* cultures, and that sodium laurate maximally inhibits acid production among saturated FA with a chain length of 8~18 (Hayes, 1984). It was also reported that laurate maximally inhibits Gram-positive organisms *in vitro* (Kabara *et al.*, 1972). Laurate acts by negative feedback so that the lower the pH the greater its inhibitory effect upon acid production by the acidogenic bacteria *S. downeii* (Hayes, 1994). Numerous reports have indicated that many surfactants act as anti-bacterial agents, producing increased membrane permeability and eventually cell lysis (Sheu and Freese, 1972; Carson and Daneo-Moore, 1980). Surfactant properties result from a combination of polar and apolar groups within the molecule. Moreover, the surface tensions of the saturated FA markedly depend on their hydrocarbon

Table 2. Effects of 1 mM medium-chain FA on polysaccharide level by standard strains of acidogenic plaque bacteria

Groups	Extracellular polysaccharide (μg/ml)	
	Water-soluble	Water-insoluble
C9[a] only	2.0 ± 0.7[d]	0
C10[b] only	0.7 ± 0.4	0
C12[c] only	1.4 ± 0.1	0
S. mutans 10449	74.6 ± 1.8	0.8 ± 0.3
S. mutans 10449 + C9	50.8 ±12.2	0.6 ± 0.2
S. mutans 10449 + C10	19.6 ± 2.3	0.4 ± 0.1
S. mutans 10449 + C12	15.5 ± 3.8	0.2 ± 0.1
S. mutans JC-2	34.4 ± 3.9	1.1 ± 0.2
S. mutans JC-2 + C9	6.2 ± 3.5	0.9 ± 0.3
S. mutans JC-2 + C10	4.4 ± 1.1	0.3 ± 0.1
S. mutans JC-2 + C12	0.6 ± 0.5	0.1 ± 0.1
S. mutans BHT	31.5 ± 3.7	0.5 ± 0.1
S. mutans BHT + C9	8.0 ± 1.1	0.3 ± 0.1
S. mutans BHT + C10	5.1 ± 1.7	0
S. mutans BHT + C12	4.1 ± 0.8	0
S. sanguis 10556	25.9 ± 4.3	0.6 ± 0.3
S. sanguis 10556 + C9	17.4 ± 8.9	0.5 ± 0.2
S. sanguis 10556 + C10	11.7 ± 1.7	0.3 ± 0.2
S. sanguis 10556 + C12	12.2 ± 0.9	0
C9 only	4.6 ± 0.2	0
C10 only	3.9 ± 0.9	0
C12 only	4.6 ± 1.3	0
L. casei ATCC 4646	43.6 ± 7.0	5.3 ± 0.5
L. casei ATCC 4646 + C9	31.9 ± 4.0	3.8 ± 0.5
L. casei ATCC 4646 + C10	25.7 ± 2.8	0.3 ± 0.2
L. casei ATCC 4646 + C12	19.2 ± 2.9	0.2 ± 0.2
L. casei ATCC 27216	67.3 ± 4.5	5 9 ± 0.4
L. casei ATCC 27216 + C9	70.1 ± 3.3	5.1 ± 0.5
L. casei ATCC 27216 + C10	40.7 ± 5.4	2 0 ± 0.2
L. casei ATCC 27216 + C12	19.0 ± 2.1	2.0 ± 0.1
C9 only	8.2 ± 0.4	0
C10 only	8.5 ± 0.5	0
C12 only	4.4 ± 0.2	0
A. viscosus ATCC 15987	222.8 ± 3.3	2.1 ± 0.2
A. viscosus ATCC 15987 + C9	241.6 ± 3.9	1.4 ± 0.5
A. viscosus ATCC 15987 + C10	98.1 ± 6.5	0.2 ± 0.2
A. viscosus ATCC 15987 + C12	25.3 ± 1.7	0.2 ± 0.1

[a]C9, sodium nonanoate.
[b]C10, sodium decanoate.
[c]C12, sodium laurate.
[d]Values represent mean ± SE from six independent determinations.

Table 3. Reductions in the sulcal caries lesions of rats maintained in a high-sucrose diet containing either sodium laurate or fluoride in drinking water

Groups[a]	Maxilla					Mandible					Total no. of teeth	Linear units (Mean±SD)
	Molar	E[b]	Ds[c]	Dm[d]	Total (n[e])	Molar	E	Ds	Dm	Total (n)		
Cariogenic diet	1st	86	0	0	86 (26)	1st	108	33	5	146 (28)		
	2nd	54	1	0	55 (27)	2nd	91	26	4	121 (28)	164	3.01 ± 2.35
	3rd	40	0	0	40 (27)	3rd	39	5	1	45 (28)		
Cariogenic diet+NaF	1st	54	0	0	54 (25)	1st	82	11	0	93 (28)		
	2nd	34	0	0	34 (25)	2nd	63	10	0	73 (28)	158	1.85 ± 1.29*
	3rd	20	1	0	21 (25)	3rd	17	0	0	17 (27)		
Cariogenic diet+sodium laurate	1st	61	1	0	62 (29)	1st	72	7	0	79 (26)		
	2nd	41	0	0	41 (29)	2nd	55	10	0	65 (26)	164	1.71 ± 1.26*
	3rd	17	0	0	17 (29)	3rd	16	0	0	16 (25)		

[a]Fifteen 4-week-old Sprague-Dawley rats were assigned to each group, each group being balanced for mean initial body weight and gender and distinguished on the basis of their diets and drinking water. Cariogenic diet group, rats that fed a mixture of foods containing 40% sucrose, 32% dry milk, 21% flour, 5% yeast, 2% anchovy, 0.01% vitamin B, and 0.01% vitamin C; cariogenic diet + NaF group, rats that fed a cariogenic diet and drinking water containing 2.64 mM NaF (50 ppm F); cariogenic diet + sodium laurate group, rats that fed a cariogenic diet containing 50 mM sodium laurate. Throughout the period of monitoring, the rats were given water ad libitum.
[b]E, enamel only.
[c]Ds. slight in dentin.
[d]Dm, moderate in dentin.
[e]n, number of teeth tested.
*Significantly different from the cariogenic diet group at $P < 0.01$.

Table 4. Proximal caries experience of rats maintained on a high-sucrose diet containing either sodium laurate or fluoride in drinking water

Groups[a]	Maxilla					Mandible					Total no. of teeth	Linear units (Mean±SD)
	Molar	E[b]	Ds[c]	Dm[d]	Total (n[e])	Molar	E	Ds	Dm	Total (n)		
Cariogenic diet	1st	1	0	0	1 (26)	1st	2	1	0	3 (28)		
	2nd	6	0	0	6 (27)	2nd	2	0	0	2 (27)	163	0.11 ± 0.34
	3rd	3	0	0	3 (27)	3rd	3	0	0	3 (28)		
Cariogenic diet+NaF	1st	2	0	0	2 (25)	1st	2	0	0	2 (28)		
	2nd	3	0	0	3 (26)	2nd	3	0	0	3 (28)	159	0.09 ± 0.27
	3rd	2	0	0	2 (25)	3rd	1	0	0	1 (27)		
Cariogenic diet+sodium laurate	1st	1	0	0	1 (29)	1st	1	0	0	1 (26)		
	2nd	7	2	0	9 (28)	2nd	2	0	0	2 (26)	163	0.10 ± 0.35
	3rd	2	0	0	2 (29)	3rd	2	0	0	2 (25)		

[a]Refer to the legend in Table 2.
[b]E, enamel only.
[c]Ds, slight in dentin.
[d]Dm, moderate in dentin.
[e]n, number of teeth tested.

chain lengths and the pH of the solution. Thus, the most effective surfactant at pH 9 is a fatty acid with a chain length of 14 (C_{14}), but this changes to C_{12} and C_{13} at pH 7, and to C_8 ~ C_{11} at pH 4 (Cooper and Zajic, 1980; Cooper *et al.*, 1981). This is probably explained by an alteration in the lipophilic/hydrophilic balance. Although we cannot conclude that the anti-caries effect of the FA is a direct function of their surfactant properties, the effect may be associated.

Many studies have revealed that dental caries is a multifactorial disease involving microflora, the host, and the diet. Therefore, the conventional measures of caries prevention are to increase the resistance of the teeth by fluoride therapy, to reduce the microbial challenge by both tooth brushing and plaque control agents, and to control diet (MacFarlane, 1989; Newbrun, 1989). Our data shows that the majority of extracellular polysaccharides, a major component of plaque matrix, produced by all strains of acidogenic plaque bacteria tested were water-soluble, and that the sodium salts of medium-chain FA with a chain length of 9~12 significantly reduced the synthesis of extracellular polysaccharides by acidogenic bacteria. Although the reason for the decreased polysaccharide level and acid production in medium-chain FA-exposed acidogenic bacteria remains unknown from this study, it may be a consequence of reduced bacterial growth in response to medium-chain FA. In fact, medium-chain FA tested notably inhibited

bacterial growth. Because decreased polysaccharide production can modify the composition of plaque bacteria and reduce plaque formation (Cole and Eastoe, 1988), it is very important to decrease bacterial metabolism. Many reports have indicated serological or genetic heterogeneity of antigenicity and virulence within bacterial strains (Kim and Chung, 1992). In addition, different antibiotic susceptibilities, serotypes, and leukotoxicities have been observed between Koreans and non-Koreans (Son *et al.*, 1985; Chung *et al.*, 1989). We thus determined the anti-caries potential of sodium laurate, which has been found to be most effective at inhibiting acid production and polysaccharide synthesis, in acidogenic plaque bacteria isolated from Korean plaque specimens. As expected, a low concentration (1 mM) of sodium laurate significantly inhibited acid production and polysaccharide synthesis in the 20 strains tested under acidic (pH 5.0) and neutral conditions *in vitro* (data not shown). Because sodium laurate showed maximum anti-caries potential *in vitro*, we further determined its anti-caries effect in the rat. In the present study, sodium laurate produced highly significant ($p < 0.01$) effects and gave a similar degree of caries prevention than fluoride. This difference was evident in sulcal caries lesions. These results are in agreement with a previous report, which showed that nonanoic acid and lauric acid significantly decrease caries occurrence in both smooth surface and sulcus of Weanling rats maintained in a high-sucrose cariogenic diets containing 2% nonanoic acid or lauric acid. When used in the mouth, laurate deprives organisms of their cariogenic property, and this could explain the anti-caries effects of FA seen in rats. A natural molecule such as laurate, which rapidly and irreversibly inhibited acid production at low pH values (Hayes, 1994), is attractive in terms of caries prevention. Fluoride is the only extensively clinically-proven measure for caries prevention (MacFarlane, 1989). It increases the acid-resistance of the teeth by incorporating fluoride in the apatite, and results in an anti-caries effect. Sodium laurate and fluoride act in an independent manner, and in terms of the carious process, suggests that sodium laurate selectively inhibits acidogenic flora, whereas fluoride probably exerted its effect both by enhancing the mineralization of early carious lesions and by increasing the resistance of the teeth to acid attack (Hayes and Stobart, 1990).

In summary, our results indicate that sodium laurate has a very strong anti-caries effect *in vitro* and *in vivo*. In addition, sodium laurate did not show any harmful side effects in experimental animals (data not shown). Since sodium laurate and fluoride acted on caries prevention in an independent manner, respectively, the use of sodium laurate combined with fluoride would probably enhance the anti-caries effect. This is supported by another study, which demonstrated that caries protection using a mixture of decanoate and fluoride on fissure caries in rats had a cumulative effect (Hayes and Stobart, 1990).

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