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Note

Antibacterial Effect of Fatty Acid Salts on Oral Bacteria

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Fatty acid salts are a type of surfactant known to have potent antibacterial activity. We therefore examined the antibacterial activities of fatty acid salts against *Streptococcus mutans*. Potassium caprylate (C10K), potassium laurate (C12K), potassium myristate (C14K), potassium oleate (C18:1K), potassium linoleate (C18:2K), and potassium linolenate (C18:3K), used at a concentration of 175 mM, resulted in a 7 log-unit reduction of *S. mutans* after a 10-min incubation. The minimum inhibitory concentration (MIC) of C18:2K and C18:3K was 5.5 mM. C12K also demonstrated high antibacterial activity (MIC of 21.9 mM). These results indicate that C12K, C18:2K, and C18:3K have high antibacterial activity against *S. mutans*, and possess great potential as antibacterial agents.

Key words：Antibacterial activity / Fatty acid salt / Oral bacteria.

Approximately 350–700 bacterial species are known to inhabit the oral cavity. Pathogenic oral bacteria can be classified into three groups: opportunistic organisms, dental caries bacteria, and gum disease bacteria. It is estimated that approximately 100 million bacteria are present in 1 mg of the bacterial structure known as plaque. Colonization of tooth surfaces by bacteria is acknowledged as the key factor in the development of dental caries and gum disease (Suido, 2011).

In this study, we focused on *Streptococcus mutans*, which is a typical member of the dental plaque community within the oral cavity and a leading cause of tooth decay. Tooth decay is caused by the destruction of the teeth by acid produced through the metabolism of dietary carbohydrates by oral bacteria. This acid production leads to a drop in local pH values resulting in tooth demineralization; thus, dental caries can be described as a loss of minerals in the teeth. Demineralization can be reversed by saliva in its early stages; re-depositing of minerals (remineralization) occurs when the pH of the plaque increases. Progression or reversal of dental caries is dependent on the balance between demineralization and remineralization, a process that is in constant flux in most people (Selwitz et al., 2007).

Fatty acid salts, a main component of soap, are the salts of a carboxylic acid chain of hydrocarbons. Fatty acid salts are a type of anionic surfactant and are produced from fatty acids and alkali. The antimicrobial effects of fatty acids have been well established. Fatty acid salts can inhibit the growth of fungi (*Aspergillus* species and *Penicillium* species) (Altieri, 2007), numerous types of bacteria (*Propionibacterium acnes, Staphylococcus aureus, Pasteurella pestis, Clostridium botulinum, Bacillus cereus*, and *Clostridium sporogenes*) (Eisler and von Metz, 1968; Ababouch et al., 1992; Chen et al., 2011; Desbois and Lawlor, 2013), and viruses (bacteriophage phi 6); (Knapp and Melly, 1986). Shapiro (1996) reported that fatty acids are effective against oral bacteria (*Porphyromonas gingivalis, Selenomonas artemidis*, and *Streptococcus sobrinus*). In addition, fatty acid salts are known to have a potent antibacterial effect on *C. botulinum, B. cereus, C. sporogenes*, and *P. pestis* (Eisler and von Metz, 1968; Ababouch et al., 1992). However, few studies

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have examined the effect of fatty acid salts on oral bacteria. In this study, we evaluated the antibacterial activity of several fatty acid salts against *Streptococcus mutans*.

A total of nine different fatty acids were used in this study: butyric acid, caproic acid (purity 98% wt, Wako Pure Chemical Industries, Ltd., Osaka, Japan); caprylic acid, capric acid (purity 98% wt, KISHIDA CHEMICAL Co., Ltd.; Osaka, Japan); lauric acid, myristic acid, oleic acid (purity 98% wt, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan); linoleic acid (purity 98% wt, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and linolenic acid (purity 70% wt, Tokyo Chemical Industry Co., Ltd.,Tokyo, Japan). Potassium hydroxide (KOH) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used as a control. Cetylpyridinium chloride (CPC) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Linear alkylbenzene sulfonate (LAS) was obtained from Shabondama Soap Co., Ltd. (Kitakyusyu, Japan).

Samples at a concentration of 350 mM were stirred for 2 h at 75℃. A KOH solution was added to obtain pH 10.5, theoretically neutralizing the fatty acid salts. All fatty acids used, including potassium butyrate (C4K), potassium caproate (C6K), potassium caprylate (C8K), potassium caprate (C10K), potassium laurate (C12K), potassium myristate (C14K), potassium oleate (C18:1K), potassium linoleate (C18:2K), potassium linolenate (C18:3K), and the KOH with a pH adjusted solution (pH 10.5) blank were filter-sterilized at a low temperature (4–6℃) using 0.20-μm Millipore filters.

Streptococcus mutans NBRC 13955 (*S. mutans*) was obtained from the NBRC (Biological Resource Center, NITE). Bacteria stored at −70℃ were thawed and incubated on trypticase soy yeast extract medium (pH 7.0–7.2) containing 30 g/l of trypticase soy broth (BD), 3 g/l of yeast extract (Difco), and 15 g/l of agar (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 37°C for 24 h. The culture solution was centrifuged at 4200 rpm for 20 min at 4℃, and the supernatant was discarded. The pellet was washed twice by resuspending it to its original volume in phosphate buffered saline (PBS; pH 7.5), followed by centrifugation and discarding of the supernatant. The pellet was finally resuspended in PBS. The spore suspension was stored at 4℃.

To test for antibacterial activity, 400 μl of fatty acid salts (final concentration of 175 mM) and 400 μl of the bacterial suspension (3.0×10^7 spores/ml) were mixed in a 1.5-ml plastic tube. A bacterial suspension mixed with KOH with a pH adjusted solution was used as the control. Samples were incubated at 25℃, for 10 min, 60 min, and 180 min; 100 μl was then plated on trypticase soy yeast extract agar medium. Plates were incubated at 37 ℃ for 2 d and then the number of colony forming units (CFU) was quantified. All experiments were conducted at least three times.

The minimum inhibitory concentration (MIC) against bacteria was determined using the two-fold dilution method. Dilutions of the fatty acid salts were mixed separately with 400 μl of *S. mutans* at 3.0×10^7 spores/ml (total volume of 800 μl), and samples were incubated for 10 min. The samples were then added to bacteria incubated at 37°C for 7 d, and spore growth was examined on trypticase soy yeast extract agar medium. The MIC was defined as the minimal antibacterial concentration that inhibited visible bacterial growth following incubation.

To examine the antibacterial activity of C12K together with other fatty acid salts (C4K, C6K, C8K, C10K, C14K, C18:1K, C18:2K, or C18:3K), C12K (21.9 mM) was mixed with either short-chain fatty acid salts (C4K, C6K), medium-chain fatty acid salts (C8K, C10K), or long-chain fatty acid salts (C14K, C18:1K, C18:2K, C18:3K), at a final concentration of either 1.75, 17.5, 35, or 87.5 mM.

Human saliva samples were collected prior to breakfast and resuspended in physiological saline (0.8% NaCl) to determine the antibacterial activity of fatty acid salts against indigenous bacteria in human saliva. Four hundred μl of the fatty acid salts (final concentration of 175 mM) was mixed with 400 μl of the saliva samples in 1.5-ml plastic tubes. A saliva sample mixed with KOH with a pH adjusted solution was used as the control. The mixtures were incubated at 25℃ at 5 min and 10 min, and 100 μl of the mixtures were plated on trypticase soy yeast extract agar medium. Plates were incubated at 37℃ for 2 d and then CFUs were quantified. All experiments were conducted at least three times.

Differences in bacterial viability from that treated by the control were assessed with a one way analysis of variance. The level of statistical significance was designated at 5 %.

Fig. 1 demonstrates the antibacterial activity of fatty acid salts against *S. mutans*. C10K, C12K, C14K, C18:1K, C18:2K, and C18:3K exhibited a 7-log reduction (log survival rate: -5±0) (99.99999% growth suppression of *S. mutans*) after 10 min of incubation. C8K displayed a 4-log reduction (log survival rate: -2± 0.7) of the units (99.99% suppression) after 180 min of incubation. C4K, C6K, and the pH adjusted control solution did not exhibit any effect even after 180 min of incubation with *S. mutans* (log survival rate: 2.0±0.2, 2.0±0.53, 1.6±0.6, respectively). The mechanism by which fatty acid salts exert their antibacterial activity remains unclear; however, the primary target seems to

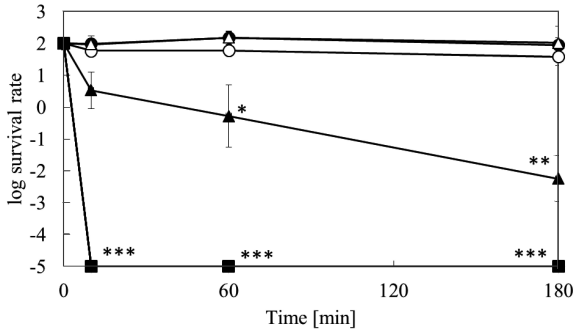

FIG. 1. The antibacterial activity of fatty acid salts (175 mM) against *S. mutans*. The log survival rate (expressed as log percentage of the survival rate of the initial CFU/ ml) was established by counting colony numbers at the time of inoculation (0 min) and after 10, 60, and 180 min of incubation. Aliquots (100 µl) of the samples were plated on trypticase soy yeast extract agar medium, and plates were incubated at 37℃ for 2 d. Symbols: ●, C4K; △, C6K; ▲, C8K; □, C10K; ■, C12K; ×, C14K; ✻, C18:1K; ◇, C18:2K; ◆, C18:3K; ○, Control (KOH with pH adjustment). Experiments were performed in triplicate, and the vertical bars represent the standard deviation. S.D. bars are not shown when masked by the graph symbol. Asterisks: significantly different ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$) from the control (one way analysis of variance)

be the bacterial cell membrane. It is hypothesized that fatty acids in solution remain sufficiently lipophilic to adsorb onto the cell surface (Ababouch et al., 1992). C4K, C6K, and C8K have weak adsorption potential since the hydrophilic group is short; thus, they are ineffective against *S. mutans*.

We subsequently examined the persistence of the effect of fatty acid salts against *S. mutans*. Fatty acid salts exhibiting high antibacterial activity (C10K, C12K, C14K, C18:1K, C18:2K, and C18:3K) were inoculated with a bacterial suspension (3.0×10^7 spores/ml), and cultured for 10 d at 37℃. No growth was apparent in any of the six fatty acid salt-bacteria cultures even after 10 d (data not shown). This suggests that the antibacterial activity of C10K, C12K, C14K, C18:1K, C18:2K, and C18:3K is long-lasting.

The determined MICs of the fatty acid salts are detailed in Table 1. The MICs of C18:2K and C18:3K were 5.5 mM. This result indicated that C18:2K and C18:3K possessed high antibacterial activity against *S. mutans*. C12K also exhibited a significant effect on *S. mutans* (MIC = 21.9 mM).

Medium-chain saturated fatty acids that lack a kinked structure can be packed more tightly. Therefore, medium-chain saturated fatty acids can reduce membrane fluidity and disrupt electron transport perhaps by restricting the movement of carriers within the membrane (Desbois, A. P., and Smith, V. J. 2010).

TABLE 1. The minimum inhibitory concentration (MIC) values of fatty acid salts against *S. mutans*.

Fatty acid salts	MIC[mM]
C4K	>175
C6K	>175
C8K	>175
C10K	87.5
C12K	21.9
C14K	>21.9
C18:1K	>21.9
C18:2K	5.5
C18:3K	5.5

Experiments were performed in triplicate.

Ababouch et al. (1992) stated that the saturated fatty acid lauric acid contain the optimal properties of water solubility and lipophilicity. Therefore, potassium laurate can easily adsorb onto the surface of cells and effectively act against *S. mutans*. The incorporation of the cis-bonds of unsaturated fatty acids into the membrane results in an increase in membrane fluidity; the cis configuration leads to kinks in the molecular shape that can develop into membrane instability (Desbois and Smith, 2010). Moreover, Zheng et al. (2005) reported that the antibacterial effect of long-chain unsaturated fatty acids was due to their inhibition of fatty acid biosynthesis. Furthermore, Knapp and Melly (1986) reported that antibacterial effects of polyunsaturated fatty acids were mediated by a peroxidative process involving H_2O_2 and bacterial iron.

Since soap is comprised of various fatty acids, and C12K exhibited the highest antibacterial activity of the saturated fatty acid salts tested, we examined the effects of fatty acid salt mixtures (C12K + another fatty acid salt) on S. *mutans*. The experimental results are shown in Table 2. As mentioned above, C4K, C6K, and C8K were ineffective against *S. mutans*; the addition of C4K, C6K, or C8K to C12K did not lead to an increase in antibacterial activity compared with that of C12K alone. Similarly, the antibacterial activity of C10, C14K, C18:1K, C18:2K, and C18:3K mixed with C12K did not differ from that of C12K on its own. These results indicate that the addition of other fatty acid salts does not inhibit the antibacterial activity of C12K. Furthermore, we examined the antibacterial activity of fatty acid salt mixtures on *S. mutans*.. The mixture of C18:2K (1/8 MIC: 0.7 mM) and C12K (1/2, 1/4, 1/8 MIC: 10.9, 5.5, 2.7 mM) did not show antibacterial synergy (Date not shown).

We compared the antibacterial activity of fatty acid salts with that of LAS or CPC. LAS is an anionic surfactant used as a household detergent (Hirasawa, 2000). CPC is a cationic surfactant used as a bactericidal agent in certain types of mouthwashes and

TABLE 2. The effect of fatty acid salt mixtures (C12K+C4K, C6K, C8K, C10K, C14K, C18:1K, C18:2K, C18:3K) on *S. mutans*.

		log survival rate						
			Concn of fatty acid salts except C12K [mM]					
Fatty acid salt mixture		initial[a]	0.175	1.75	17.5	35.0	87.5	175 (not C12K)
	C4K	2.00	—[b]	—	—	—	—	1.99±0.25
	C6K	2.00	—	—	—	—	—	1.98±0.19
	C8K	2.00	—	—	—	—	—	0.53±0.58
C12K +	C10K	2.00	—	—	—	—	—	—
(21.9mM)	C14K	2.00	—	—	—	—	—	—
	C18:1K	2.00	—	—	—	—	—	—
	C18:2K	2.00	—	—	—	—	—	—
	C18:3K	2.00	—	—	—	—	—	—

The log survival rate (expressed as log percentage of the survival rate of the initial CFU/ml) was established by counting colony numbers at the time of inoculation (0 min) and after 10 min of incubation. Aliquots (100 µl) of the samples were plated on trypticase soy yeast extract agar medium, and plates were incubated at 37℃ for 2 d. Values are mean (± S.D.) of three replicates.

[a] initial bacterial suspension

[b] no viable cells

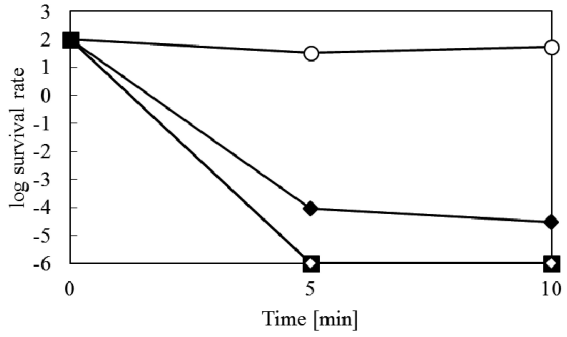

FIG. 2. The antibacterial activity of 175 mM C12K, C18:2K or C18:3K against oral bacteria present in the human saliva (initial bacterial concentration, 6.5×10^8 CFU/ml). The log survival rate (expressed as the log percentage of the survival rate of the initial CFU/ml) was established by counting colony numbers at the time of inoculation (0 min) and after 5 and 10 min of incubation. Aliquots (100 µl) of the samples were plated on trypticase soy yeast extract agar medium, and plates were incubated at 37℃ for 2 d. Symbols: ■, C12K; ◇, C18:2K; ◆, C18:3K; ○, Control (KOH with pH adjustment). Experiments were performed in triplicate.

toothpastes (Suido, 2011). LAS and CPC exhibited a 7 log-unit antibacterial effect after 10 min of incubation (data not shown); the MICs of LAS and CPC were 2.7 mM and 0.7 mM, respectively (data not shown). The MICs of fatty acid salts C18:2K and C18:3K against *S. mutans* were comparable to those of LAS and CPC, suggesting that the antibacterial activity of C18:2K and C18:3K was equivalent to that of currently utilized bactericidal agents.

The oral cavity is colonized by many other microorganisms such as *Streptococcus sobrinus, Porphyromonas gingivalis, Actinomyces israelii, Prevotella intermedia, Actinomyces naselundii*, and *Candida albicans* (Suido, 2011). Therefore, we examined the antibacterial activity of fatty acid salts (C12K, C18:2K, C18:3K) against oral bacteria present in human saliva. The experimental results are shown in Fig. 2. C12K and C18:2K demonstrated an 8 log-unit reduction after 10 min of incubation, while C18:3K exhibited a 6 log-unit reduction of after 10 min of incubation. In the present study, we have demonstrated that the MICs of C18:2K and C18:3K against *S. mutans* were 5.5 mM. Moreover, the MICs of C12K against S. mutans was 21.9 mM. However, C12K and C18:2K demonstrated high antibacterial activity against oral cavity bacteria. It is reported that C12 had a wide antibacterial spectrum compared with C18:2 and C18:3 (KABARA et al., 1972). Therefore, it is assumed that C12K exhibited high antibacterial activity against oral cavity bacteria after a 5-min incubation since C12K had a wide antibacterial spectrum compared with C18:2K and C18:3K.

These results indicate that C12K, C18:2K, and C18:3K are effective against oral bacteria present in human saliva, and that C12K, C18:2K, and C18:3K possess great potential as an oral care agent (mouthwash). Improving the oral cavity flora by removing gum disease bacteria and opportunistic organisms is of more significance than inhibiting all oral bacteria. Further studies are need to assess whether fatty acid salts are useful in improving the oral cavity flora.

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