

The antibacterial activity of various saturated and unsaturated fatty acids against several oral pathogens

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Abstract

The antibacterial activity of various saturated fatty acids (SFA) and unsaturated fatty acids (USFA) against different oral pathogens which are implicated in the cause of dental caries, stomatitis, gingivitis, and periodontitis was examined. The saturated fatty acids Pa, StA and ArA, and the unsaturated ω -7 fatty acids PLA and ω -9 fatty acids OA showed either none to low antimicrobial activity against all of the 12 oral pathogenic strains used in this study. In contrast, the ω -3 PUFAs, ALA, SDA, EPA and DHA, and the ω -6 PUFAs, LA, GLA, and AA showed considerable antimicrobial activity against 8, 7, 6 and 5 strains, and 6, 10 and 5 strains, respectively. In particular, the ω -3 and ω -6 PUFAs showed strong antimicrobial activity against *Porphyromonas gingivalis* KCTC 381, the cause of periodontitis, and against *Aggregatibacter segnis* KCTC 5968, *Fusobacterium nucleatum* subsp. *Polymorphum* KCTC 5172 and *Prevotella intermedia* KCTC 25611, all organisms implicated in the cause of gingivitis. To date, no bacterial resistance to free fatty acids has been encountered and no resistance phenotype has emerged. Therefore, these results suggest that PUFAs may be useful in the development of therapeutic agents for oral diseases, and in particular, in the development of agents that have minimal side effects and against which there is no bacterial resistance.

Key words

Antimicrobial activity, Free fatty acid, Oral pathogen

Introduction

Systemic or topical antibiotics have been used as an adjunct in the treatment of periodontal disease (Slots and Ting, 2002). *Prevotella* and *Porphyromonas* species, including the main oropharyngeal pathogenic species, have traditionally been considered susceptible to penicillin. However, a gradual increase in the rate of resistance to penicillin has been noted by several investigators over the recent years (Appelbaum, 1992). At present, the antibiotics used most frequently against anaerobic bacteria include metronidazole, imipenem, and meropenem. These are active against almost all clinically-relevant anaerobic bacteria, although strains resistant to these agents have been reported sporadically (Falagas and Siakavellas, 2000;

Aldridge *et al.*, 2001). Thus, many researchers have sought to develop new therapeutic agents for periodontal disease with reduced side effects and high antibacterial activity.

The antibacterial activity of long-chain unsaturated fatty acids has been well-known for many years. Fatty acids function as the key ingredients of antimicrobial food additives which inhibit the growth of unwanted microorganism. Additionally, the long-chain unsaturated fatty acids such as linoleic and oleic acids are bactericidal to important pathogenic microorganisms, including methicillin-resistant *Staphylococcus aureus* (Farrington *et al.*, 1992), *Helicobacter pylori* (Hazell and Graham, 1990), and *Candida albicans* (Bergsson *et al.*, 2001). In recent years, it has been reported that EPA also exhibited

antimicrobial activity against *Bacillus subtilis*, *Listeria monocytogenes*, *S. aureus* and *Pseudomonas aeruginosa* (Shin et al., 2007).

Even though there have been several reports on the antibacterial activity of unsaturated fatty acids against different many human pathogens, the antibacterial activity of unsaturated fatty acids against oral pathogens has not been confirmed. Therefore, the aim of this study was to measure the antibacterial activity of various saturated and unsaturated fatty acids against the oral pathogens implicated in the etiology of dental caries, stomatitis, gingivitis, and periodontitis.

Materials and Methods

Free fatty acids: The commercially available fatty acids were tested for antimicrobial activity against several oral pathogens. The saturated fatty acids (SFA), palmitic acid (PA), stearic acid (StA) and arachidic acid (ArA) and the unsaturated fatty acid (USFA), palmitoleic acid (PLA), oleic acid (OA), linoleic acid (LA), α -linolenic acid (ALA), arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were purchased of analytical grade (98-99%) from Sigma and Fluka to measure their *in vitro* antimicrobial activities.

Test microorganisms and culture media: Twelve strains of the microorganism used in this study were obtained from the Korean Collection for Type Culture (KCTC; Deajeon, Korea). *Streptococcus mutans* KCTC 3065 and KCTC 3289 (the major cause of dental caries), *Candida albicans* KCTC 17485 and *C. albicans* var. *albicans* KCTC 17484 (the major cause of stomatitis), *Aggregatibacter actinomycetemcomitans* KCTC 3698, *Aggregatibacter segnis* KCTC 5968, *Fusobacterium nucleatum* subsp. *vincenti* KCTC 5105, *F. nucleatum* subsp. *polymorphum* KCTC 5172, and *Prevotella intermedia* KCTC 5506 and KCTC 25611 (the major causes of gingivitis), and *Porphyromonas gingivalis* KCTC 5352 and KCTC 381 (the major cause of periodontitis) were used in this study. All these microorganisms were cultured following the method of Choi et al. (2012).

Determination of MIC values: The antimicrobial activity of free fatty acids was confirmed using broth microdilution susceptibility tests to measure the Minimal Inhibitory Concentration (MIC). The antimicrobial agent chloramphenicol (Sigma C1919) was included in the assays as a positive control. All the MIC tests were performed independently and in triplicate.

Results and Discussion

MIC values of the saturated fatty acids: The MIC values of PA and StA were above 100 $\mu\text{g ml}^{-1}$ against all 12 oral pathogenic strains; these fatty acids exhibited none to low

antimicrobial activity. ArA also showed none to low antimicrobial activity except against *Streptococcus mutans* KCTC 3065 and *Aggregatibacter actinomycetemcomitans* KCTC 3698 (Table 1).

MIC values of ω -7 and ω -9 MUFAs: With the exception of *Porphyromonas gingivalis* KCTC 381, the unsaturated ω -7 fatty acid PLA showed no antibacterial activity when tested against all the oral pathogens. The unsaturated ω -9 fatty acid OA showed significant levels of antimicrobial activity only against *Prevotella intermedia* KCTC 25611 and 5506 (Table 2).

MIC values of ω -3 and ω -6 PUFAs: PUFAs were divided into the ω -3 PUFA group of PUFAs (ALA (C18:3 n-3), SA (C18:4 n-3), EPA (C20:5 n-3) and DHA (C22:6 n-3)), and into the ω -6 PUFA group of PUFAs (LA (C18:2 n-6), GLA (C18:3 n-6) and AA (C20:4 n-6)) according to their double-bond position. In the testing against the 12 strains, the ω -3 PUFAs, ALA, SDA, EPA and DHA showed considerable antimicrobial activity against 8, 7, 6 and 5 strains, respectively. In particular, all the ω -3 PUFAs showed strong antimicrobial activity against the cause of periodontitis, *P. gingivalis* KCTC 381, and the cause of gingivitis, *Aggregatibacter segnis* KCTC 5968, *Fusobacterium nucleatum* subsp. *Vincenti* KCTC 5105, *Fusobacterium nucleatum* subsp. *Polymorphum* KCTC 5172 and *P. intermedia* KCTC 25611. When tested against 12 strains, the ω -6 PUFAs, LA, GLA, and AA showed considerable antimicrobial activity against 6, 10 and 5 strains, respectively. Notably, all ω -6 PUFAs showed strong antimicrobial activity against the cause of periodontitis, *P. gingivalis* KCTC 381, and the cause of gingivitis, *A. segnis* KCTC 5968, *F. nucleatum* subsp. *Polymorphum* KCTC 5172 and *P. intermedia* KCTC 25611. The MIC values of chloramphenicol, the positive control, were in the range of 1.22-625 $\mu\text{g ml}^{-1}$ when tested against all of the 12 strains (Table 2).

There are numerous reports on the antibacterial activity of fatty acids against human pathogens, including fungi and yeast, but only few contain data regarding the inhibitory effects against oral pathogens, a major cause of dental caries, stomatitis, gingivitis, and periodontitis (Newman et al., 2003; Choi et al., 2012).

In the antibacterial assay (Table 1 and 2), unsaturated fatty acids showed greater inhibition than saturated fatty acids, which is consistent with the results reported by several other investigators. In previous reports, the antibacterial action of fatty acids is usually attributed as being a property of the long-chain unsaturated fatty acids, including oleic acid, linoleic acid, and linolenic acid, while long-chain saturated fatty acids, including palmitic acid and stearic acid, are reported as showing less antibacterial activity (Sun et al., 2003; Seidel and Taylor 2004). This

Table 1 : The MIC value ($\mu\text{g ml}^{-1}$) of chloramphenicol as a positive control, and the MIC values of the free saturated fatty acids against 12 oral pathogens which cause dental caries, stomatitis, periodontitis and gingivitis. All the MIC tests were performed independently and in triplicate

Fatty acid		S. mu 3289	S. mu 3065	C. al 17484	C. al 17485	P. gi 5352	P. gi 381
Chloramphenicol		9.76	625	625	625	2.44	2.44
PA	C16:0	>1,250	>1,250	312.50	>1,250	>1,250	625
StA	C18:0	>1,250	>1,250	312.50	>1,250	>1,250	2,500
ArA	C20:0	>1,250	78.12	312.50	>1,250	312.50	>1,250
Fatty acid		A. ac 3698	A. se 5968	F. nu 5105	F. nu 5172	P. in 25611	P. in 5506
Chloramphenicol		4.88	2.44	1.22	1.22	1.22	1.22
PA	C16:0	>1,250	>1,250	>1,250	>1,250	>1,250	>1,250
StA	C18:0	>1,250	>1,250	>1,250	>1,250	>1,250	>1,250
ArA	C20:0	78.12	156.25	>1,250	>1,250	>1,250	>1,250

S. mu 3289: *Streptococcus mutans* KCTC 3289; S. mu 3065: *Streptococcus mutans* KCTC 3065; C. al 17484: *Candida albicans* var. *albicans* KCTC 17484; C. al 17485: *Candida albicans* KCTC 17485; P. gi 5352: *Porphyromonas gingivalis* KCTC 5352; P. gi 381: *Porphyromonas gingivalis* KCTC 381; A. ca 3698: *Aggregatibacter actinomycetemcomitans* KCTC 3698; A. se 5968: *Aggregatibacter segnis* KCTC 5968; F. nu 5105: *Fusobacterium nucleatum* subsp. *Vincentii* KCTC 5105; F. nu 5172: *Fusobacterium nucleatum* subsp. *Polymorphum* KCTC 5172; P. in 25611; *Prevotella intermedia* KCTC 25611; P. in 5506; *Prevotella intermedia* KCTC 5506

Table 2 : The MIC value ($\mu\text{g ml}^{-1}$) of the free unsaturated fatty acids against several oral pathogens involved in the etiology of dental caries, stomatitis, periodontitis and gingivitis. All the MIC tests were performed independently and in triplicate

Fatty acid		S. mu 3289	S. mu 3065	C. al 17484	C. al 17485	P. gi 5352	P. gi 381
PLA	C16:1(n-7)	>1,250	312.50	>1,250	>1,250	>1,250	39.06
OA	C18:1(n-9)	1,250	>1,250	>1,250	625	>1,250	625
ALA	C18:3(n-3)	19.53	>1,250	625	312.50	19.53	9.76
SDA	C18:4(n-3)	156.25	1,250	39.06	312.50	312.50	9.76
EPA	C20:5(n-3)	9.76	625	625	1,250	78.12	9.76
DHA	C22:6(n-3)	>1,250	625	1,250	1,250	312.50	9.76
LA	C18:2(n-6)	19.53	>1,250	156.25	312.50	78.12	9.76
GLA	C18:3(n-6)	19.53	625	1,250	78.12	78.12	9.76
AA	C20:4(n-6)	>1,250	625	625	625	312.50	9.76
Fatty acid		A. ac 3698	A. se 5968	F. nu 5105	F. nu 5172	P. in 25611	P. in 5506
PLA	C16:1(n-7)	>1,250	>1,250	>1,250	>1,250	>1,250	>1,250
OA	C18:1(n-9)	>1,250	>1,250	2,500	312.50	78.12	9.76
ALA	C18:3(n-3)	625	9.76	9.76	78.12	19.53	9.76
SDA	C18:4(n-3)	312.50	39.06	39.06	39.06	39.06	39.06
EPA	C20:5(n-3)	312.50	9.76	9.76	2.44	39.06	312.5
DHA	C22:6(n-3)	625	19.53	39.06	39.06	78.12	>1,250
LA	C18:2(n-6)	1,250	19.53	312.50	39.06	39.06	>1,250
GLA	C18:3(n-6)	78.12	9.76	9.76	19.53	39.06	9.76
AA	C20:4(n-6)	625	19.53	39.06	39.06	19.53	312.50

tendency was also observed in the present study

As shown in Table 2, all the ω -3 and ω -6 PUFAs used in this study exhibited a comparatively broad range of antimicrobial activity against the tested oral pathogens, and among them, GLA exhibited the broadest antimicrobial range. In the study, where large number of pathogenic strains were examined, strains that caused periodontitis and gingivitis

appeared to be more susceptible to antibacterial action of the ω -3 and ω -6 PUFAs than the strains that caused dental caries and stomatitis. Moreover, to date, no bacterial resistance to free fatty acids has been encountered and no resistance phenotype has emerged (Desbois *et al.*, 2009). In addition, the fatty acids of SA (Khan *et al.*, 2007), GLA (Kapoor and Huang, 2006) EPA and DHA (Hasturk *et al.*, 2006) displayed anti-inflammatory properties.

In conclusion, for the first time in this study it has been reported that free fatty acids inhibit the growth of oral pathogens. Thus, this study presents a strategy for the prevention and/or treatment of oral infection using therapies that have minimal side effects, and against which no bacterial resistance is shown.

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