Original Article

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Inhibitory effects of several saturated fatty acids and their related fatty alcohols on the growth of *Candida albicans*

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Summary **We examined the effect of 5 saturated fatty acids and their related alcohols on the growth of *Candida albicans*. The inhibitory effects of these compounds against the yeast and hyphal growth forms of *C. albicans* were examined using the modified NCCLS method and crystal violet staining, respectively. Among these compounds, capric acid inhibited both types of growth at the lowest concentration. The IC_{80}, *i.e.*, the concentration at which the compounds reduced the growth of *C. albicans* by 80% in comparison with the growth of control cells, of capric acid for the hyphal growth of this fungus, which is indispensable for its mucosal invasion, was 16.7 μM. These fatty acids, including capric acid, have an unpleasant smell, which may limit their therapeutic use. To test them at reduced concentrations, the combined effect of these fatty acids and oligonol, a depolymerized polyphenol, was evaluated *in vitro*. These combinations showed potent synergistic inhibition of hyphal growth [fractional inhibitory concentration (FIC) index = 0.319]. Our results demonstrated that capric acid combined with oligonol could be used as an effective anti-*Candida* compound. It may be a candidate prophylactic or therapeutic tool against mucosal *Candida* infection.**

Keywords: Medium-chain fatty acid, capric acid, oligonol, *Candida albicans*

1. Introduction

Candida albicans, a dimorphic fungus, is a member of the oral and intestinal microbial flora in healthy human individuals. Its excessive growth can cause pathological symptoms such as oral, esophageal, vaginal, or systemic candidiasis (*1,2*). Recently, it was suggested that heavy colonization by *C. albicans* predisposes to various types of inflammatory diseases (*3*). There are several types of foods that can control *Candida* growth *in vitro* and *in vivo*, for example, lemongrass, green tea, and cassia (*4*). Consuming foods with anti-*Candida* activity may prevent the excessive growth of *C. albicans*. It has been reported that medium-chain fatty acids have anti-*Candida* activity (*5*). These fatty acids might be the functional food components for the improvement of symptoms related to *Candida* overgrowth. We have previously demonstrated that capric acid is an active component responsible for the anti-*Candida* activity of *Houttuynia cordata* (*6*).

In the present study, we systematically examined the effects of several saturated fatty acids and their related fatty alcohols on the growth of *C. albicans*. We demonstrated that capric acid could be used in anti-*Candida* treatment and might be a candidate prophylactic or therapeutic tool against mucosal *Candida* infection.

2. Materials and Methods

2.1. *C. albicans strain*

We used *C. albicans* strain TIMM1768, a clinically isolated serotype A strain (Teikyo University Institute of Medical Mycology, Tokyo, Japan).

2.2. *Medium-chain fatty acids, their related fatty alcohols, and oligonol*

Medium-chain fatty acids and related fatty alcohols were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). They were dissolved in dimethyl sulfoxide (DMSO) at 10% w/w before dilution with

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RPMI-1640 medium (RP medium). Oligonol, a low-molecular-weight polyphenol formulation derived from lychee fruit (Amino Up Chemical Co., Ltd.), was diluted with RP medium for *in vitro* experiments.

2.3. *Inhibitory effects of the compounds against C. albicans yeast growth*

The inhibitory effects of several saturated fatty acids and their related fatty alcohols against *C. albicans* yeast growth were determined using the microbroth dilution assay recommended by NCCLS M-27-A (7). *C. albicans* cells were cultured in YPG medium (1% Bacto-peptone, 0.5% yeast extract, 2% glucose, pH 6.5) for 16 h at 37°C with shaking at 38 rpm. The cells were collected and washed twice with RP medium, and the cell suspension was prepared in the same medium at 1×10^4 cells/mL. Medium-chain fatty acids and their related fatty alcohols in DMSO and DMSO control samples were diluted with RP medium. Mixtures of 100 µL of *Candida* cell suspension and 100 µL of various compound dilutions in DMSO (or control) were placed in a 96-well microplate. The microplate was incubated for 24 h at 30°C. Then, the minimum inhibitory concentration (MIC) values were determined.

2.4. *Inhibitory effects of the compounds against C. albicans hyphal growth*

RP medium supplemented with 2.5% heat-inactivated fetal calf serum, 20 mM HEPES, 2 mM L-glutamine, and 16 mM sodium hydrogen carbonate (pH 7.0) was used as the hyphal growth-promoting medium for *C. albicans*. *C. albicans* suspension was prepared at 5×10^3 cells/mL. Each well of a 96-well flat-bottom microplate received a mixture of 100 µL of *Candida* suspension, 100 µL of fatty acid or fatty alcohol preparations, or 50 µL of fatty acid or fatty alcohol preparation or oligonol preparations. The plates were incubated at 37°C in a 5% CO_2 atmosphere for 15 h. To determine the extent of *C. albicans* hyphal growth, the crystal violet (CV) staining assay was performed as described previously (*8*). In brief, the medium from the wells was discarded and the adhering *Candida* mycelia were sterilized with 70% ethanol. The mycelia were stained with 0.01% CV and washed with water. The microplates were dried and 150 µL of isopropanol containing 0.04 N HCl and 50 µL of 0.25% sodium dodecyl sulfate were added to the wells and mixed. The absorbance at 620 nm (triplicate samples) was measured spectrophotometrically. MIC was defined as the lowest compound concentration that reduced growth by 80% or 85% in comparison with the growth in the drug-free well.

To analyze the combined anti-*Candida* activities, the fractional inhibitory concentration (FIC) index was calculated as follows: FIC = [(A)/MICA] + [(B)/MICB], where MICA and MICB are the MICs of samples A and B, respectively, determined separately. (A) and (B) are the concentrations of the samples in combination, respectively, in all of the wells corresponding to an MIC (isoeffective combinations) (*9*). FIC indices were used to characterize antibiotic interactions as follows: synergy, FIC index ≤ 0.5; additivity, 0.5 < FIC index < 1; indifference, 1 < FIC index ≤ 4; and antagonism, FIC index > 4.

3. Results

3.1. *Inhibitory effects of saturated fatty acids and their related fatty alcohols on C. albicans yeast growth*

The inhibitory effects of 5 saturated fatty acids and 4 fatty alcohols against *C. albicans* yeast growth were examined using the modified NCCLS method (Table 1). The MICs of octanoic acid, capric acid, and lauric acid against yeast growth were 34.7 mM, 29.0 mM, and 49.9 mM, respectively. However, $C_{8\text{-}12}$ alcohols 1-octanol, decanol, and dodecanol did not affect the growth of *Candida* cells at concentrations below 200 mM. C_4 acids and C_{14} acid and alcohol (sodium butyrate, myristic acid, and 1-tetradecanol) did not significantly affect yeast growth at concentrations below 100 mM. Thus, medium-chain fatty acids showed a stronger inhibitory effect than short- and long-chain fatty acids. The inhibitory properties of related alcohols were weak.

3.2. *Inhibitory effects of saturated fatty acids and fatty alcohols on C. albicans hyphal growth*

The inhibitory effects of various saturated fatty acids and fatty alcohols against the growing hyphae of *C. albicans* were examined using the CV staining method (Table 2). Most of the tested compounds significantly inhibited the hyphal growth of the fungus at very low concentrations.

The inhibitory effects of 5 saturated fatty acids and 4 fatty alcohols were compared in terms of their IC_{80} values, *i.e.*, the concentration at which the compounds reduced the growth of *C. albicans* by 80% in comparison with the growth of control cells. The IC_{80} of capric acid and lauric acid was 16.7 µM and 61.0 µM, respectively. These inhibitory concentrations were approximately 1/1,000 of the MIC for yeast growth (Tables 1 and 2). However, 205 µM octanoic acid was needed for 80% inhibition of *C. albicans* hyphal growth. The IC_{80} of 1-octanol was almost the same as that of octanoic acid (175 µM). The IC_{80} of decanol (204 µM) and dodecanol (401 µM) was approximately 10 times higher than that of capric and lauric acid. The IC_{80} of myristic acid was 833 µM. Sodium butyrate did not inhibit *Candida* hyphal growth at concentrations below 1.82×10^3 µM.

These results show that the inhibitory effects of

Table 1. Effect of saturated fatty acids and their related fatty alcohols on the total growth

C	Fatty acid	MIC (mM)	Fatty acid alcohol	MIC (mM)
4	Sodium n-Butyrate	363	–	–
8	n-Octanoic acid	34.7	1-Octanol	307
10	Capric acid	29.0	Decanol	> 253
12	Lauric acid	49.9	Dodecanol	> 215
14	Myristic acid	> 175	1-Tetradecanol	> 187

Activities were measured using the modified NCCLS method as described in the Materials and Methods section. The minimum inhibitory concentration (MIC) against *Candida* growth is shown.

Table 2. Effect of several saturated fatty acids and their related fatty alcohols on *C. albicans* hyphal growth

C	Fatty acid	IC_{80} (μM)	Fatty alcohol	IC_{80} (μM)
4	Sodium n-Butyrate	$> 1.82 \times 10^3$	–	–
8	n-Octanoic acid	205	1-Octanol	175
10	Capric acid	16.7	Decanol	204
12	Lauric acid	61.0	Dodecanol	401
14	Myristic acid	833	1-Tetradecanol	> 930

Activities were measured using the CV staining method as described in the Materials and Methods section. The concentration causing 80% inhibition (IC_{80}) of *Candida* hyphal growth is indicated.

Table 3. IC_{85} and FIC index for medium-chain fatty acids or their related alcohols in combination with oligonol against *Candida* hyphal growth

Items	IC_{85} (μM)	IC_{85} with Oligonol 62.5 μg/mL	FIC index
Capric acid	14.5	2.90	0.319
Lauric acid	32.0	13.5	0.541
Decanol	106	68.8	0.800
Dodecanol	440	123	0.432

Activities were measured using the CV staining method. The concentration causing 85% inhibition (IC_{85}) of *Candida* hyphal growth using a combination of medium-chain fatty acids or alcohols and/or oligonol (62.5 μg/ml) is indicated. The FIC index was calculated as described in the Materials and Methods section.

C_{10} and C_{12} acids against *Candida* hyphal growth were exceptionally strong compared with the inhibitory effects against *Candida* yeast growth. This inhibition was approximately 10 times stronger than the effect of C_{10} and C_{12} alcohols.

3.3. *Inhibition of C. albicans hyphal growth by saturated fatty acids or their related fatty alcohols in combination with a low-molecular-weight polyphenol*

It has been reported that a combination of capric acid and terpinen-4-ol, a major component of tea tree oil, inhibits *Candida* hyphal growth synergistically (*10*). We have also reported that oligonol, a low-molecular-weight polyphenol formulation derived from lychee fruit, inhibits *Candida* hyphal growth (*11*). The preparation has attained a self-affirmed Generally Recognized as Safe (GRAS) status in the USA, which supports its safety as a food product. Here we examined the inhibitory effect of a combination of C_8-C_{12} acids or alcohols and oligonol against *C. albicans* hyphal growth. Their combined effect was evaluated in terms of reduction of the IC_{85} value and the FIC index (Table 3).

In the case of capric acid alone, 14.5 μM concentration was needed for 85% inhibition (IC_{85}) of *Candida* hyphal growth (Figure 1A). However, the IC_{85} of capric acid administered in combination with oligonol (62.5 μg/mL) decreased to approximately 1/5 of this value (2.90 μM) (Figure 1A). The IC_{85} of lauric acid when combined with oligonol was reduced to approximately half of the value obtained when it was used alone (13.5 μM). Figure 1B shows the concentrations of capric acid and oligonol in combination showing 85% inhibition of *Candida* growth. The curve, located under the dotted line, indicates that the combined effect was synergistic. The data in Table 3 shows that the combination of capric acid (3.50 μM) and oligonol (31.3 μg/mL) displayed synergistic activity (FIC index = 0.319). The FIC index of lauric acid with oligonol slightly exceeded 0.500. Using dodecanol alone, a concentration of 440 μM was needed for 85% inhibition of *Candida* hyphal growth (30 times higher than the IC_{85} of capric acid). The IC_{85} of dodecanol decreased to 1/4 on combination with oligonol; a synergistic effect was observed (FIC index = 0.432).

These results indicated that capric acid and dodecanol with oligonol effectively repressed *Candida* hyphal growth.

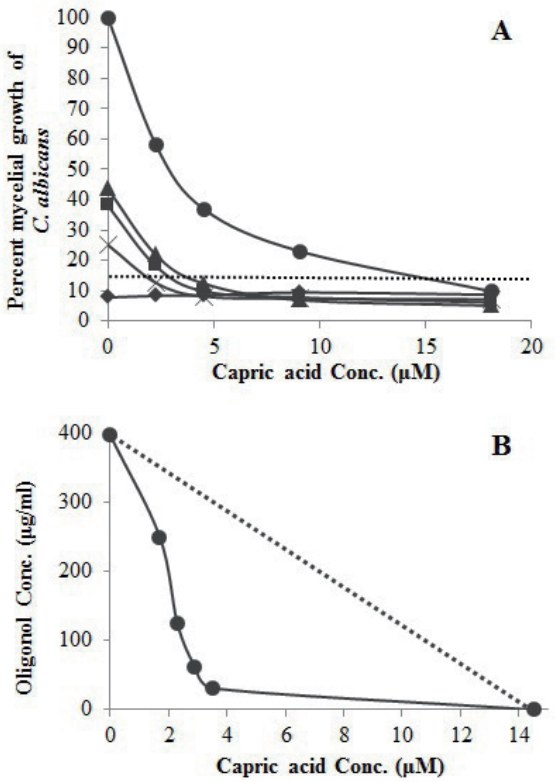

Figure 1. *Candida* growth in a medium containing various concentrations of capric acid and/or oligonol. (A) *C. albicans* cells (TIMM1768) were cultured in a medium containing a combination of the indicated concentrations of capric acid and 0 (●), 31.3 (▲), 62.5 (■), 250 (×), or 500 (♦) μg/mL of oligonol for 15 h (dotted line = IC_{85}). (B) Analysis of the combined effect. Each point represents the concentration of the combination of capric acid and oligonol causing 85% inhibition of *C. albicans* hyphal growth. If the combined effect was additive, the point for the combination would lie on the dotted line.

4. Discussion

It has been reported that a low concentration of capric acid inhibits *C. albicans* hyphal growth *in vitro* and that oral administration of approximately 10 mg/mL (50 μL) of capric acid protects mice from oral candidiasis (*12*). These data suggest that capric acid may be used as a functional food with anti-*Candida* activity. However, capric acid has a characteristic unpleasant smell; therefore, it might not be suitable for oral administration. To find a better candidate for oral use, we examined the anti-*Candida* activity of other fatty acids and their related alcohols. The results clearly showed that among the tested compounds, capric acid inhibited *C. albicans* yeast and hyphal growth at the lowest concentration. This result demonstrates that capric acid is the most suitable candidate for protection against mucosal candidiasis. Davis *et al.* (*13*) have reported that dodecanol (C_{12} alcohol) effectively represses *Candida* hyphal growth. Here we also confirmed that straight-chain fatty alcohols inhibited hyphal growth but their effective concentrations (C_{10}, C_{12}) were much higher than the required concentrations of the related carbonic acids (Table 2). Therefore, we speculated that the effects of decanol and dodecanol could be mediated by their metabolic acids, capric and lauric acid, respectively.

To decrease the effective doses of capric acid for anti-*Candida* function, the inhibitory effect of the combination of capric acid and oligonol, a low-molecular-weight polyphenol formulation derived from lychee fruits, was tested. Polyphenols are likely to be some of the best compounds for such combinations; they have antimicrobial activity not only against *C. albicans* but also against *Helicobacter pylori* (*14*), *Staphylococcus aureus*, and *Escherichia coli* O157:H7 (*15*). By combining capric acid and lauric acid with oligonol, their IC_{85} values for inhibition of *C. albicans* hyphal growth were lowered to 3-14 μM. This result suggests that these fatty acids can function as effective anti-*Candida* compounds in the presence of polyphenols.

It would be useful to find out whether medium-chain fatty acids affect *C. albicans* growth in the human digestive tract. The concentration of medium-chain fatty acids in the gastrointestinal tract has not been examined thoroughly. However, it has been reported that approximately 50% of the total amount of medium-chain fatty acids infused into the duodenum gradually moves into the blood circulation within 3 h (*16*). This observation suggests that the medium-chain fatty acids in the gastrointestinal tract maintain their concentration at significant levels at least for a 3-h period. In Japan, the daily intake of medium-chain fatty acids is approximately 0.2 g. If a meal contains 0.02 g (1/10 of the daily intake) of medium-chain fatty acids and it arrives in the 100-cm^3 duodenum, the concentration in the duodenum will be approximately 1 mM. In this study, 1 mM medium-chain fatty acids could not inhibit *C. albicans* yeast growth *in vitro* but inhibited hyphal growth. We consider that medium-chain fatty acids, perhaps as metabolites of glycerides, have the potential to elicit their anti-*Candida* activity in the duodenum or small intestine, particularly in the presence of polyphenols.

The mechanism of inhibition of *Candida* hyphal growth by the combination of capric acid and oligonol is not clear. However, the inhibitory effect of dodecanol and catechin in the *Candida* hyphal growth pathway has been partially explained. Dodecanol exerts its effect through a mechanism involving enhanced expression of the *C. albicans* hyphal repressor Sfl1p (*17*). Catechin inhibits *C. albicans* dimorphism by suppressing Cek1 phosphorylation and cAMP synthesis (*18*). In our experiments, the combination of dodecanol and oligonol showed a synergistic inhibitory effect on *C. albicans* hyphal growth. We can speculate that the synergistic inhibitory effect of capric acid and oligonol might reflect complex interactions at different points in the pathway of hyphal growth.

In this study, a very low concentration of capric acid inhibited *C. albicans* hyphal growth. The intake of some neutral fats, such as coconut oil, composed of medium-chain fatty acids may inhibit the overgrowth of *C. albicans* in the gut. Medium-chain fatty acids are the products of fat degradation by lipase in the gut. We found that coconut oil (500 μg/mL) was degraded by lipase within 30 min, and its 10-fold diluted solution inhibited approximately 50% of *Candida* hyphal growth (data not shown). Future studies should examine the role of foods containing medium-chain fatty acids in the dynamic regulation of the ecology of *C. albicans* in our intestinal ducts, particularly in combination with other vegetable foods containing polyphenols.

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