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

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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...
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 × 10⁶ 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 × 10³ 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% CO₂ 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, C₁₀ 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 IC₈₀ 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 IC₈₀ 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 IC₈₀ of 1-octanol was almost the same as that of octanoic acid (175 μM). The IC₈₀ of decanol (204 μM) and dodecaneol (401 μM) was approximately 10 times higher than that of capric and lauric acid. The IC₈₀ of myristic acid was 833 μM. Sodium butyrate did not inhibit Candida hyphal growth at concentrations below 1.82 × 10³ μM.

These results show that the inhibitory effects of
concentration was needed for 85% inhibition (IC\textsubscript{85}) of \textit{Candida} hyphal growth (Figure 1A). However, the IC\textsubscript{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\textsubscript{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 \textit{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 \textit{Candida} hyphal growth (30 times higher than the IC\textsubscript{85} of capric acid). The IC\textsubscript{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 \textit{Candida} hyphal growth.

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

### 3.3. Inhibition of \textit{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 \textit{Candida} hyphal growth synergistically (10). We have also reported that oligonol, a low-molecular-weight polyphenol formulation derived from lychee fruit, inhibits \textit{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\textsubscript{8}-C\textsubscript{12} acids or alcohols and oligonol against \textit{C. albicans} hyphal growth. Their combined effect was evaluated in terms of reduction of the IC\textsubscript{80} value and the FIC index (Table 3).

In the case of capric acid alone, 14.5 μM concentration was needed for 85% inhibition (IC\textsubscript{85}) of \textit{Candida} hyphal growth (Figure 1A). However, the IC\textsubscript{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\textsubscript{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 \textit{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 \textit{Candida} hyphal growth (30 times higher than the IC\textsubscript{85} of capric acid). The IC\textsubscript{85} of dodecanol decreased to 1/4 on combination with oligonol; a synergistic effect was observed (FIC index = 0.432).

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reported that dodecanol (C12 alcohol) effectively inhibited mucosal candidiasis. Davis et al. (13) have reported that dodecanol (C12 alcohol) effectively represses Candida hyphal growth. Here we also confirmed that straight-chain fatty alcohols inhibited hyphal growth but their effective concentrations (C10, C12) 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 IC85 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-cm3 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 SNF1p (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.

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 (C12 alcohol) effectively represses Candida hyphal growth. Here we also confirmed that straight-chain fatty alcohols inhibited hyphal growth but their effective concentrations (C10, C12) were much higher than the required concentrations

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**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 = IC85). (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.
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.

References


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