In vitro antimicrobial properties of caprylic acid, monocaprylin, and sodium caprylate against *Dermatophilus congolensis*

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**Objective**—To determine antimicrobial effects of caprylic acid and its derivatives, monocaprylin and sodium caprylate, on *Dermatophilus congolensis* and to determine effects of caprylic acid on the ultrastructure of *D congolensis* by use of transmission electron microscopy (TEM).

**Sample**—3 strains of *D congolensis* (33411, 33413, and 14639).

**Procedures**—Strains of *D congolensis* were incubated separately under anaerobic conditions at 37°C for up to 48 hours in brain heart infusion (BHI) broth that was supplemented with various concentrations of caprylic acid (7.5, 12.5, 15, 17.5, or 20 mM), monocaprylin (2.5, 5, 7.5, or 10 mM), or sodium caprylate (15, 50, 60, 70, 100, or 120 mM) or contained no antimicrobial treatment. After incubation, bacterial counts were determined by means of plating in triplicate on BHI-agar plates. Caprylic acid–treated or untreated *D congolensis* samples were embedded in epoxide resin for TEM; cross sections were examined for structural damage.

**Results**—Minimum inhibitory concentrations of caprylic acid, monocaprylin, and sodium caprylate against *D congolensis* were 7.5, 2.5, and 15 mM, respectively. Minimum bactericidal concentrations of caprylic acid, monocaprylin, and sodium caprylate against *D congolensis* were 15, 5, and 70 mM, respectively. Examination via TEM revealed that a 15-mM concentration of caprylic acid disintegrated the plasma membrane of *D congolensis*.

**Conclusions and Clinical Relevance**—Results indicated that caprylic acid, monocaprylin, and sodium caprylate could potentially be used to treat *D congolensis* infections. However, in vivo studies should be undertaken to determine whether these compounds can be considered as treatment options. (Am J Vet Res 2011;72:331–335)
In the study reported here, we investigated the antimicrobial effects of caprylic acid, monocaprylin, and sodium caprylate on *D. congolensis* in vitro to examine the potential of these products for future application in the treatment of dermatophilosis. The objectives of the present study were to determine the MIC and MBC of caprylic acid, monocaprylin, and sodium caprylate against 3 strains of *D. congolensis* (33411, 33413, and 14639). Additionally, the bactericidal kinetics of these molecules on *D. congolensis* and the effect of the MIC and MBC of caprylic acid on *D. congolensis* at the ultrastructural level were investigated.

**Materials and Methods**

**Bacterial cultures**—*Dermatophilus congolensis* (strains 33411, 33413, and 14639) were individually incubated under anaerobic conditions (85% N₂, 10% CO₂, and 5% O₂) at 37°C for 48 hours in 10-ml volumes of sterile BHI broth. After incubation, bacterial cells were sedimented via centrifugation (3,600 x g for 15 minutes at 4°C), washed twice with PBSS (sterile; pH, 7.2), and resuspended in PBSS. Portions (0.1 mL) of appropriate dilutions to achieve < 10⁵ CFUs/mL of each cell suspension were added to BHIA plates, and plates were incubated anaerobically at 37°C for 48 hours to identify and quantify the bacterial population in each culture. The upper and lower limits of reliable quantification for these plates were 20 and 200 CFUs/plate, respectively. Cells were resuspended in PBSS as previously described at dilutions necessary to obtain the desired concentration of inoculum (5 log₁₀ CFUs/mL).

**MIC and MBC determination**—The MIC and MBC of caprylic acid, monocaprylin, and sodium caprylate against *D. congolensis* were determined via broth dilution assay as described in another study. Tubes containing BH broth supplemented with sterile solutions of caprylic acid (concentrations, 0 to 20mM), monocaprylin (0 to 10mM), or sodium caprylate (0 to 120mM) were inoculated separately with *D. congolensis* at final concentrations of 5.0 log₁₀ CFUs/mL and incubated anaerobically at 37°C for 48 hours. Tubes of *D. congolensis*-inoculated BH broth devoid of caprylic acid and its derivatives were included as controls. After incubation, the samples were serially diluted (1:10) via addition of PBSS, and 10² to 10⁴ CFUs of *D. congolensis* were surface-plated on BHIA plates. After plates were incubated anaerobically at 37°C for 48 hours, colonies were counted. The lowest concentration of antimicrobial treatment that caused visibly detectable inhibition of *D. congolensis* growth was accepted as the MIC of that treatment. The lowest concentration of each treatment that prevented growth of the organism when subcultured on BHIA plates after serial dilution and plating was accepted as the MBC. Duplicate samples were included for each treatment at each concentration, and each experiment was replicated 3 times.

**Bactericidal kinetics**—A 200-µL suspension of *D. congolensis* strain 33411 (concentration, 5.0 log₁₀ CFUs/mL) was added to approximately 9.8 mL of BHI broth that contained the MIC, MBC, or more than the MBC of caprylic acid (< 20mM), monocaprylin (< 10 mM), or sodium caprylate (< 120 mM) to bring the final volume to 10 mL. Control samples containing *D. congolensis*-inoculated BH broth with no added antimicrobial treatment were also included. Samples were incubated anaerobically at 37°C for up to 48 hours. Colonies of *D. congolensis* were counted at the time of inoculation (0 hours) and after 6, 12, 24, and 48 hours of incubation by means of plating 0.1-mL portions of the samples (undiluted or after serial dilutions 1:10) in PBSS on triplicate BHIA plates anaerobically at 37°C for 48 hours. When no bacterial colonies were detected in plates of undiluted samples, the samples were tested for surviving bacteria by enriching 1 mL of the sample in 100 mL of BHI broth anaerobically at 37°C for 48 hours. If any turbidity was noted in the broth, the culture was streaked on BHIA plates, and these were examined for grayish-white, moist, smooth mucoid colonies typical of *D. congolensis*. Duplicate samples were included for each treatment and control. Triplicate plates of each sample were used to quantify the bacterial population, and each experiment was replicated 3 times.

**TEM**—*Dermatophilus congolensis* cultures that contained the MIC or MBC of caprylic acid (7.5 and 15mM, respectively) or contained no caprylic acid were incubated anaerobically for 6 hours (15mM caprylic acid) or 48 hours (all others) at 37°C in 10 mL of BHI broth. After incubation, cultures were rinsed twice with PBSS (pH, 7.2), resuspended in 5 mL of PBSS, and pelleted by centrifugation at 5,000 x g for 5 minutes. Pellets were fixed in PBSS containing 1.5% glutaraldehyde and 1.5% formaldehyde solution for 1 hour and washed 3 times (20 minutes each) in PBSS on ice as described elsewhere. Additional fixation was performed by use of a solution of 1.0% osmium tetroxide and 0.8% potassium ferricyanide in PBSS, refrigerated at 4°C for 1 hour. Fixed pellets were washed 3 times for 10 minutes each in cold distilled water, followed by gradual dehydration by washing in 30%, 50%, 70%, 95%, and 100% propylene oxide at 23°C. After dehydration, each pellet was infiltrated with a 1:1 mixture of resin and propylene oxide with a 3:1 mixture of resin and propylene oxide with 1.5% DMP 30 accelerator and each sample was cured at 60°C for 42 hours. Several 80- to 90-nm-thick, ultrathin sections of the pellets were obtained by use of a glass knife. Sections were stained with 4% uranyl acetate and 2.3% lead citrate and examined by use of an electron microscope.

**Statistical analysis**—Data from 3 independent replicate trials performed on duplicate samples were analyzed to study bacterial kinetics via a mixed procedure by use of ANOVA. Since no significant effects of replication were noted, replication was not included in further analysis. The model statement included 5 treatments for each compound, 5 time periods (0, 6, 12, 24, and 48 hours), and all interactions of treatment and time. The model statement used to analyze the experiments for MIC and MBC included 5 treatments of...
monocaprylin, 6 treatments each of caprylic acid and sodium caprylate, and 2 time periods (0 and 48 hours). Experimental units were the inoculation tubes from which multiple samples were obtained over time. Inoculation tubes within treatment were defined as random effects. The least significant difference test with appropriate corrections for multiple comparisons was used to determine significant ($P < 0.01$) difference between the means.

**Results**

A 7.5 mM concentration of caprylic acid inhibited the growth of all 3 strains of *D. congolensis* (33411, 33413, and 14639) after 48 hours of culture; there was no significant ($P > 0.01$) difference between bacterial counts at 0 hours in untreated control tubes and after 48 hours in samples that contained a 7.5 mM concentration of caprylic acid. Monocaprylin inhibited the growth of all 3 targeted bacterial strains at a concentration of 2.5 mM. Similarly, *D. congolensis* counts in samples that contained a 15 mM concentration of sodium caprylate were not significantly ($P > 0.01$) different after 48 hours of culture from those of control samples at 0 hours. Bacteria were not detectable (<1 CFUs/mL) after the 48-hour incubation period with 15, 5, and 70 mM concentrations of caprylic acid, monocaprylin, and sodium caprylate, respectively. On the basis of these results, the respective MIC and MBC were determined as 7.5 and 15 mM for caprylic acid, 2.5 and 5 mM for monocaprylin, and 15 and 70 mM for sodium caprylate. The 3 tested strains of *D. congolensis* were most sensitive to monocaprylin, followed by caprylic acid and sodium caprylate.

The survival curves of *D. congolensis* exposed to various concentrations of caprylic acid, monocaprylin, and sodium caprylate were determined (Figures 1–3). The mean bacterial count in treatment and control samples at 0 hours was approximately 6 log$_{10}$ CFUs/mL. In control samples, *D. congolensis* counts increased significantly by 1 log$_{10}$ CFUs/mL after 48 hours of incubation, compared with the values at 0 hours; as expected, bacterial counts in samples that contained the MIC of caprylic acid or of its derivatives did not differ significantly from the initial count at 0 hours. However, *D. congolensis* counts were decreased significantly ($P < 0.01$) after 48 hours of culture from counts determined at 0 hours in samples that were prepared by the addition of caprylic acid, monocaprylin, or sodium caprylate at or above the MBC. A > 3-log reduction in counts of *D. congolensis* (where 1 log decrease represents a 10-fold decrease in bacterial concentration) was detected in samples that contained the MBC of caprylic acid or its derivatives.

A variety of cell configurations of *D. congolensis* were detected via TEM, although the ultrastructures of these cells were essentially the same among the different samples. Control samples of *D. congolensis* cells not exposed to caprylic acid or its derivatives were essentially coccoid cells that had intact plasma membranes.
with clearly visible nucleosomes and ribosomes (Figure 4). However, the plasma membranes of *D. congolensis* cells treated with 7.5mM caprylic acid appeared shrunk, with wavy projections toward the periplasmic space (Figure 5). The width of periplasmic space in these cells was found to be increased (indicative of incipient plasmolysis) in comparison with that of control cells. In *D. congolensis* samples treated with 15mM caprylic acid, extensive damage of the plasma membrane was observed in which the membrane was disjointed and fragmented (Figure 6).

**Discussion**

At the present time, antimicrobial drugs are commonly administered for the treatment and control of dermatophilosis. The development of bacterial drug resistance associated with the use of antimicrobial drugs in animal agriculture has emerged as an important global public health concern. It is known that bacteria are capable of multiple mutations that may be selected for via such antimicrobial use. Many drugs, including erythromycin, spiramycin, penicillin G, ampicillin, chloramphenicol, streptomycin, amoxicillin, tetracyclines, and novobiocin, were reported to be effective in the treatment of dermatophilosis. In contrast, resistance against polymyxin B, enrofloxacin, oxacillin, neomycin, and trimethoprim-sulfamethoxazole was reported in a few isolates of *D. congolensis*. The present study investigated in vitro activity of caprylic acid and its derivatives against dermatophilosis. Researchers have suggested that emergence of resistance against the activities of FFAs and monoglycerides is unlikely because these target biological membranes. Moreover, FFAs and their derivatives were suggested to interfere with antimicrobial resistance mechanisms in bacteria via inhibition of the signal transduction process. It was also proposed that some FFAs inhibit amino acid uptake by diffusing into the bacterial cell and lowering the pH of the protoplasm, thereby disrupting the normal activities of intracellular enzymes.

Previously, caprylic acid was reported to be effective in killing herpes simplex virus, respiratory syncytial virus, haemophilus influenza, group B streptococci, major mastitis-causing bacteria such as staphylococcus, and *E. coli*. Monocaprylin was highly effective against pathogenic bacteria, including *E. coli* O157:H7 and *L. monocytogenes*. Sodium caprylate was proven to be highly effective against *E. coli* O157:H7 in the drinking water of cattle.

Results of the present study indicated that caprylic acid, monocaprylin, and sodium caprylate are effective in killing *D. congolensis* and that monocaprylin is the most effective in vitro molecule on the basis of concentrations. The results of examination via TEM indicated that exposure to caprylic acid damages the plasma membrane of *D. congolensis* and causes leakage of cellular contents to the exterior, which is likely the means of bacterial destruction. Since monocaprylin and caprylic acids are lipophilic, these could potentially be applied to the skin of affected animals in an ointment formulation. Sodium caprylate is a water-soluble compound and may potentially be applied in a topical disinfectant solution to clean the lesions associated with derma-
tophilosis. However, further in vivo studies should be conducted to ascertain the effectiveness and safety of these compounds.

a. American Type Culture Collection, Manassas, Va.
c. Sigma-Aldrich, St Louis, Mo.
d. SPI Supplies / Structure Probe Inc, West Chester, Pa.
e. Polysciences Inc, Warrington, Pa.
g. SAS, version 8.0, SAS Institute Inc, Cary, NC.

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