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Antibacterial Activity of Sphingoid Bases and Fatty Acids against Gram-Positive and Gram-Negative Bacteria

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There is growing evidence that the role of lipids in innate immunity is more important than previously realized. How lipids interact with bacteria to achieve a level of protection, however, is still poorly understood. To begin to address the mechanisms of antibacterial activity, we determined MICs and minimum bactericidal concentrations (MBCs) of lipids common to the skin and oral cavity—the sphingoid bases D-sphingosine, phytosphingosine, and dihydrosphingosine and the fatty acids sapienic acid and lauric acid—against four Gram-negative bacteria and seven Gram-positive bacteria. Exact Kruskal-Wallis tests of these values showed differences among lipid treatments ($P < 0.0001$) for each bacterial species except *Serratia marcescens* and *Pseudomonas aeruginosa*. D-Sphingosine (MBC range, 0.3 to 19.6 μg/ml), dihydrosphingosine (MBC range, 0.6 to 39.1 μg/ml), and phytosphingosine (MBC range, 3.3 to 62.5 μg/ml) were active against all bacteria except *S. marcescens* and *P. aeruginosa* (MBC > 500 μg/ml). Sapienic acid (MBC range, 31.3 to 375.0 μg/ml) was active against *Streptococcus sanguinis*, *Streptococcus mitis*, and *Fusobacterium nucleatum* but not active against *Escherichia coli*, *Staphylococcus aureus*, *S. marcescens*, *P. aeruginosa*, *Corynebacterium bovis*, *Corynebacterium striatum*, and *Corynebacterium jeikeium* (MBC > 500 μg/ml). Lauric acid (MBC range, 6.8 to 375.0 μg/ml) was active against all bacteria except *E. coli*, *S. marcescens*, and *P. aeruginosa* (MBC > 500 μg/ml). Complete killing was achieved as early as 0.5 h for some lipids but took as long as 24 h for others. Hence, sphingoid bases and fatty acids have different antibacterial activities and may have potential for prophylactic or therapeutic intervention in infection.

Common sphingolipids and fatty acids are involved in the physical barrier, permeability barrier, and immunologic barrier functions of the skin and oral mucosa (8, 14). Epithelial layers contain ceramides, free fatty acids, and cholesterol; sebaceous lipids at the skin surface include a complex mixture of triglycerides, fatty acids, wax esters, squalene, cholesterol, and cholesterol esters; and saliva contains the same sebaceous lipids (6, 14, 19). These sebaceous secretions contribute to (i) the transport of fat-soluble antioxidants to the skin and mucosal surfaces, (ii) the pro- and anti-inflammatory properties of the skin and mucosal surfaces, and (iii) the innate antimicrobial activity of the skin and mucosal surfaces (20, 26, 27).

Although the composition, biosynthesis, secretion, and function of cutaneous lipids are well characterized from extensive and elegant work done in the 1970s, little is known about their role in controlling microbial infection and colonization. Certain fatty acids and sphingoid bases found at the skin and mucosal surfaces are known to have antibacterial activity and are thought to play a more direct role than previously thought in innate immune defense against epidermal and mucosal bacterial infections (10). They include free sphingosines, dihydrosphingosines, lauric acid, and sapienic acid. In human subjects, for example, the number of *Staphylococcus aureus* CFU per unit area of skin is inversely proportional to both the sapienic acid content and the free sphingosine content (1, 22). The lowest concentrations of both these antimicrobial lipids were found in subjects with atopic dermatitis, for whom *S. aureus* infections are frequently a problem.

More recently, the same lipids have been shown to be present in the oral cavity, in saliva and at mucosal surfaces (5, 6). The fatty acids are derived from sebaceous triglycerides, while sphingoid bases are derived from epithelial sphingolipids through the action of hydrolytic enzymes.

In this study, we hypothesized that the sphingoid bases D-sphingosine, dihydrosphingosine, and phytosphingosine and the fatty acids sapienic acid and lauric acid, commonly found on the skin and in mucosa, have antimicrobial activity against Gram-positive and Gram-negative bacteria found on the skin and in the oral cavity. We also suggest potential mechanisms for lipid antimicrobial activity and present their potential as pharmaceuticals to improve therapies for treatment and control of a wide variety of cutaneous and mucosal infections and inflammatory disorders.

MATERIALS AND METHODS

Bacterial species and growth conditions. Bacteria commonly found in the skin and oral microbiomes were used (13, 25). *Escherichia coli* and *Serratia marcescens* were also included to obtain information about typical Gram-negative bacterial susceptibility and resistance. *E. coli* ATCC 12795, *S. aureus* ATCC 29213, *S. marcescens* ATCC 14756, and *Pseudomonas aeruginosa* ATCC 47085 were grown for 3 h in Mueller-Hinton broth (Difco Laboratories, Detroit, MI) at 37°C. *Corynebacterium bovis* ATCC 7715, *Corynebacterium striatum* ATCC 7094, and *Corynebacterium jeikium* ATCC 43734 were grown for 3 h in brain heart infusion broth (Difco Laboratories, Detroit, MI) supplemented with 0.1% Tween 80 (ICN Biomedicals, Aurora, OH) at 37°C in an atmosphere containing 5% CO_2. *Streptococcus sanguinis* ATCC 10556 and *Streptococcus mitis* ATCC 6249 were grown for 3 h in tryptic soy broth (Difco Laboratories, Detroit, MI) supplemented with 0.6% yeast extract (Difco Laboratories, Detroit,

Received 23 June 2011 Returned for modification 18 August 2011
Accepted 6 December 2011
Published ahead of print 12 December 2011
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Supplemental material for this article may be found at http://aac.asm.org/.

doi:10.1128/AAC.05151-11

TABLE 1 MICs, MBCs, and median MBCs of sphingoid bases and fatty acids for Gram-negative and Gram-positive bacteria[a]

Parameter	Value[b]						*P* value
	D-Sphingosine	Phytosphingosine	Dihydrosphingosine	Lauric acid	Sapienic acid	SMAP28[c]	
E. coli							
MIC mean	7.8 ± 0.0	3.9 ± 0.0	15.6 ± 0.0	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	1.7 ± 0.7	
MBC mean	19.6 ± 13.6	15.6 ± 0.0	39.1 ± 15.6	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	ND	
MBC median	19.6	15.6	31.3	ND[d]	ND[d]	ND	$P < 0.0001$[e]
P. aeruginosa							
MIC mean	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	1.6 ± 0.6	
MBC mean	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	ND	
MBC median	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND	
S. marcescens							
MIC mean	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	3.3 ± 1.4	
MBC mean	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	>500.0 ± 0.0[d]	ND	
MBC median	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND	
F. nucleatum							
MIC mean	0.7 ± 0.2	3.3 ± 0.7	2.0 ± 0.2	2.1 ± 1.0	6.5 ± 1.3	0.6 ± 0.1	
MBC mean	4.9 ± 2.0	3.9 ± 0.0	2.0 ± 0.0	6.8 ± 2.0	86.0 ± 46.9	ND	
MBC median	3.9	3.9	2.0	7.8	93.8	ND	$P < 0.0001$[e]
S. aureus							
MIC mean	1.3 ± 0.3	1.6 ± 0.3	1.3 ± 0.3	250.0 ± 0.0	>500.0 ± 0.0[d]	3.3 ± 1.3	$P < 0.0001$[e]
MBC mean	1.3 ± 0.5	7.8 ± 0.0	4.7 ± 3.7	250.0 ± 0.0	62.5 ± 0.0	ND	
MBC median	1.0	7.8	4.9	250.0	62.5	ND	
S. sanguinis							
MIC mean	2.0 ± 0.0	7.8 ± 0.0	0.7 ± 0.0	10.4 ± 2.6	52.1 ± 10.4	5.0 ± 0.0	
MBC mean	1.3 ± 0.5	3.4 ± 1.0	1.3 ± 0.5	125.0 ± 0.0	31.3 ± 0.0	ND	
MBC median	1.0	3.9	1.0	125.0	31.3	ND	$P < 0.0001$[e]
S. mitis							
MIC mean	0.5 ± 0.2	7.8 ± 3.9	0.3 ± 0.0	15.6 ± 0.0	140.2 ± 20.8	5.0 ± 0.0	
MBC mean	0.2 ± 0.0	3.0 ± 1.1	0.3 ± 0.2	15.6 ± 11.1	375.0 ± 144.3	ND	$P < 0.0001$[e]
MBC median	0.2	3.0	0.2	11.7	375.0	ND	
C. bovis							
MIC mean	1.6 ± 0.3	5.2 ± 1.3	5.2 ± 1.3	416.7 ± 83.3	>500.0 ± 0.0[d]	0.5 ± 0.2	
MBC mean	15.6 ± 0.0	62.5 ± 0.0	15.6 ± 0.0	156.3 ± 62.5	>500.0 ± 0.0[d]	ND	
MBC median	15.6	62.5	15.6	125.0	ND[d]	ND	$P < 0.0001$[e]
C. striatum							
MIC mean	1.3 ± 0.3	4.2 ± 1.3	1.0 ± 0.0	250.0 ± 0.0	>500.0 ± 0.0[d]	0.02 ± 0.0	
MBC mean	2.0 ± 0.0	7.8 ± 0.0	2.0 ± 0.0	375.0 ± 144.3	>500.0 ± 0.0[d]	ND	
MBC median	2.0	7.8	2.0	375.0	ND[d]	ND	$P < 0.0001$[e]
C. jeikeium							
MIC mean	5.2 ± 1.3	13.0 ± 5.2	10.4 ± 2.6	208.3 ± 41.7	>500.0 ± 0.0[d]	0.03 ± 0.0	$P < 0.0001$[e]
MBC mean	11.7 ± 4.5	31.3 ± 0.0	15.6 ± 0.0	93.8 ± 36.1	>500.0 ± 0.0[d]	ND	
MBC median	11.7	31.3	2.0	93.8	ND[d]	ND	

[a] Gram-negative bacteria include *E. coli*, *P. aeruginosa*, *S. marcescens*, and *F. nucleatum*. Gram-positive bacteria include *S. aureus*, *S. sanguinis*, *S. mitis*, *C. bovis*, *C. striatum*, and *C. jeikeium*.

[b] Mean MIC and MBC (μg/ml) ± standard deviation. ND, not done.

[c] SMAP28 was used as a positive assay control to show that the microdilution assays were set up properly and that MICs were accurate and within previously reported ranges. MBC determinations were not completed, and the results were not included in statistical analyses.

[d] MICs or MBCs are larger than the upper limit of detection for the assay.

[e] Significance at the 0.05 level. Significance probabilities are associated with the nonparametric Kruskal-Wallis test of the null hypothesis that the distribution of MBCs is the same across all treatment groups with a specified bacterial species.

MI) at 37°C in an atmosphere containing 5% CO_2. *Fusobacterium nucleatum* ATCC 25586 was grown in Schaedler's broth (Difco Laboratories, Detroit, MI) for 3 h at 37°C in an anaerobic Coy Chamber (Coy Laboratory Products Inc., Grass Lake, MI). Before use, all bacterial cell suspensions were adjusted to contain 1×10^8 CFU/ml (optical density at 600 nm [OD_{600}], 0.108; Spectronic 20D+; Thermo Fisher Scientific, Inc., Waltham, MA) and diluted with appropriate media to 10^7 CFU/ml (*F. nucleatum*), 10^6 CFU/ml (*S. mitis*), or 10^5 CFU/ml (remaining bacteria).

Preparation of lipids. D-Sphingosine, phytosphingosine, dihydrosphingosine, and lauric acid were obtained from Sigma Chemical Company (St. Louis, MO). Sapienic acid was obtained from Matreya Inc. (Pleasant Gap, PA). The lipids were dissolved in a chloroform-methanol solution (2:1), and purity was confirmed by thin-layer chromatography. The lipids, dried under nitrogen, were then suspended in sterile 0.14 M NaCl to make a 1.0-mg/ml stock solution, sonicated in 5-min increments to suspend the lipid, and diluted to the desired concentration using 0.14 M NaCl.

Antimicrobial assays. Broth microdilution assays were used to determine the MIC (defined as the lowest concentration of lipid that reduced growth by more than 50%) and the MBC (defined as the lowest concentration of lipid that prevented growth) of each lipid for each bacterium (15, 24). Briefly, lipid suspensions were diluted in 0.14 M NaCl (500 to 1 μg/ml) in microtiter plates (Immunolon 1 microtiter plates; Thomas Scientific, Swedesboro, NJ). Bacterial cultures in their respective concentra-

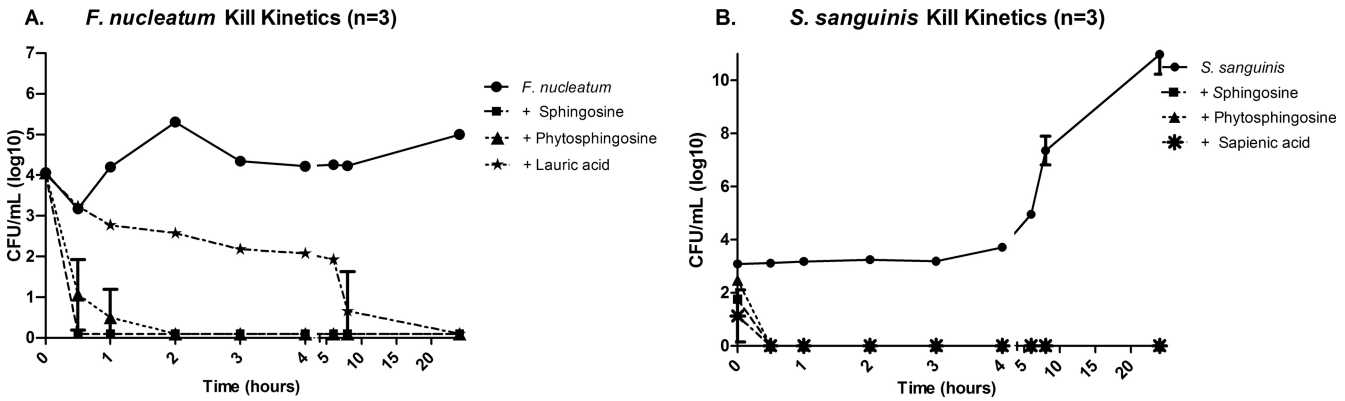

FIG 1 Kinetic killing of select bacteria with lipid treatments at 10 times the MIC. Where no bacteria were recovered, +1 was added to the zero values before log transformation of the data. A geometric mean of $n = 3$ is shown for each data point. The error bars show standard errors of the mean (SEM). (A) *F. nucleatum* with D-sphingosine, phytosphingosine, and lauric acid. (B) *S. sanguinis* with D-sphingosine, phytosphingosine, and sapienic acid. Additional kill kinetics are shown in Fig. S1C to F in the supplemental material.

tions and media were then added. Medium without microorganisms was added to 0.14 M NaCl in wells used as the plate blank negative controls. Medium with microorganisms was added to 0.14 M NaCl in wells used as the plate growth positive controls. After appropriate incubation times, the optical density of bacterial growth was read in the spectrophotometer (Spectromax Microplate Reader; Molecular Devices Corp., Sunnyvale, CA), and the MIC was determined. At higher concentrations, the lipids had an optical density that interfered with the determination of an MIC. Therefore, MBCs were also derived by plating bacteria from the completed broth microdilution assays onto 5% sheep blood agar plates (Remel, Lenexa, KS) and examining for the presence of colonies. MIC and MBC determinations were repeated in quadruplicate.

SMAP28 [RGLRRLGRKIAHGVKKYGPTVLRIIRIA-(NH_2)] was synthesized as previously described (15) by NeoMPS, Inc. (San Diego, CA) and suspended in 0.14 M NaCl. SMAP28 was included in this study as a positive control to show that the microdilution assay was set up properly and that MICs were accurate and within previously reported ranges. SMAP28 is effective against Gram-positive bacteria, Gram-negative bacteria, and fungi but not against some corynebacteria (15).

Killing kinetics assays. Killing kinetics assays were performed using the spiral-plating method (11). For this, a 3-h culture of each bacterial suspension, adjusted to the appropriate concentration for each bacterium (as described above), was split among five groups, and each was mixed with either 0.14 M NaCl (negative control) or lipids at a concentration equivalent to 10× the MIC determined in the broth microdilution assays. At time intervals of 0, 0.5, 1, 2, 3, 4, 6, 8, and 24 h, 1-ml samples of treated bacteria and controls were removed, serially diluted into 0.14 M NaCl, and plated onto 5% sheep blood agar plates (Remel, Lenexa, KS) using an Autoplate 4000 Automated Spiral Plater (Advanced Instruments, Inc., Norwood, MA). The plates were incubated appropriately, colonies were counted using standard spiral-plater methodology, and concentrations were calculated. Killing kinetics assays were repeated in triplicate.

Statistical analyses. The exact Kruskal-Wallis test was employed to detect differences in the MICs and MBCs, utilizing a 5% level of statistical significance. This nonparametric analog to analysis of variance (ANOVA) was used due to modest sample sizes and violations of the normality assumptions for parametric procedures. Significance probabilities are for the test of the null hypothesis that the distribution of outcome values is the same for all the treatment groups designated. *Post hoc* pairwise comparisons were not performed due to modest sample sizes.

Two measures of killing kinetics were computed and analyzed. The trapezoidal area under the concentration-time curve (AUC) was used as a summary measure of bacterial variability over the treatment time course, and comparisons were made with and without the inclusion of the AUCs from the control sample. The significance probabilities reported are associated with the null hypothesis that the distribution of the trapezoidal area is the same among the specified treatment groups. A second summary measure of killing kinetics over time considered was time to zero, defined as the first time point at which total bacterial counts reached zero. Note that for certain of these longitudinal assays (i.e., from a given vial), none of the bacterial counts in the series reached zero. In such instances, the value of the corresponding time to zero was assigned the highest rank for purposes of analysis. If several such instances occurred in a given analysis, ties for the highest rank were assigned.

RESULTS

Sphingoid bases and fatty acids had antimicrobial activity for a variety of Gram-positive and Gram-negative bacteria. MIC, MBC (Table 1), and kinetic killing curves (Fig. 1A and B; see Fig. S1C to F in the supplemental material) clearly showed that some sphingoid bases and fatty acids were more potent for some microbial species than for others. For example, sphingoid bases were antimicrobial for two of the four Gram-negative organisms tested, *E. coli* and *F. nucleatum* (MIC range, 0.7 to 15.6 μg/ml), while fatty acids were active only for *F. nucleatum* (MIC range, 2.1 to 6.5 μg/ml). Kinetic assays showed that killing of *E. coli* and *F. nucleatum* with sphingosine and phytosphingosine occurred within 0.5 to 2 h (Table 2), whereas killing of *F. nucleatum* with lauric acid was more gradual and occurred within 24 h. Time-to-zero outcomes indicated significant differences among lipid treatments for *F. nucleatum* ($P = 0.0143$). SMAP28 was used as a positive assay control, and MIC values ranged from 0.1 μg/ml for *C. striatum* and *C. jeikeium* to 10.0 μg/ml for *S. marcescens.*

Also, sphingoid bases were antimicrobial for all six of the Gram-positive bacteria (MIC range, 0.3 to 13.0 μg/ml) (Table 1), and fatty acids were more active for oral streptococcus species (MIC range, 10.4 to 140.2 μg/ml) than *S. aureus* (MIC range, 250 to >500 μg/ml). Of the fatty acids, only lauric acid was weakly antibacterial for *C. bovis*, *C. striatum*, and *C. jeikeium* (MIC range, 208.3 to 416.7 μg/ml). Kinetic assays showed that killing of *S. aureus*, *S. sanguinis*, *S. mitis*, and *C. striatum* with sphingosine and phytosphingosine occurred within 0.5 to 6 h (Table 2) but killing of *S. aureus* with lauric acid and killing of *S. sanguinis* and *S. mitis* with sapienic acid was gradual and occurred within 24 h. Time-to-zero outcome comparisons indicated significant differences among lipid treatments for *S. mitis* and *C. striatum* ($P = 0.0036$ for each).

TABLE 2 Time-to-zero comparisons[a]

Bacterium	Treatment lipids compared	Median time to zero (h)	*P* value[b]
E. coli	Phytosphingosine	2.0	0.10
	Sphingosine	0.5	
F. nucleatum	Phytosphingosine	1.0	0.0143[c]
	Sphingosine	0.5	
	Sapienic Acid	8.0	
S. aureus	Phytosphingosine	24.0	0.10
	Sphingosine	1.0	
S. sanguinis	Phytosphingosine	0.5	1.00
	Sphingosine	0.5	
	Sapienic Acid	0.5	
S. mitis	Phytosphingosine	24.0	0.0036[c]
	Sphingosine	6.0	
	Lauric Acid	1.0	
C. striatum	Phytosphingosine	3.0	0.0036[c]
	Sphingosine	4.0	
	Lauric Acid	0.5	

[a] Time-to-zero comparisons for each bacterial species are shown, along with significance probabilities for each compared group. Comparisons were made without the control group, as the control samples did not produce zero values.
[b] Significance probabilities are associated with the exact nonparametric Kruskal-Wallis test of the null hypothesis that the distribution of time to zero is the same across all treatment groups with a specified bacterial species.
[c] Significance at the 0.05 level.

Exact Kruskal-Wallis tests confirmed differences among the lipid treatments ($P < 0.0001$) for each of the bacterial species, with the exception of *S. marcescens* and *P. aeruginosa.* Comparisons of the trapezoidal AUCs also showed significant differences among all treatment lipids for each of the organisms ($P < 0.004$ in all instances). When controls were omitted from the analysis, significant differences were seen among all the lipid treatments compared, except for *E. coli*, where there was no evidence that the AUC distributions differed for phytosphingosine and sphingosine (Table 3).

It is also worth noting that when bacteria were suspended in a simple saline solution, kill kinetics assays were vastly different (data not shown). Complete killing of *E. coli* and *S. aureus*, suspended in 0.14 M NaCl with phytosphingosine occurred within 0.5 h. This was a reduction of 3×10^4 CFU/ml for *E. coli* and 2×10^4 CFU/ml for *S. aureus.*

DISCUSSION

Lipids typically found on the skin and in mucosa have antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria found on the skin and in the oral cavity. In this study, we show that the sphingoid bases D-sphingosine, phytosphingosine, and dihydrosphingosine, as well as the two fatty acids sapienic acid and lauric acid, had variable antimicrobial activities for a variety of Gram-positive and Gram-negative bacteria. These results are similar to those of others who have shown that sphingosine, dihydrosphingosine, and phytosphingosine are active against *Candida albicans* (4) and that fatty acids and their monoglycerides are antimicrobial for group A and group B streptococcus (2, 10, 17, 23).

Although the exact mechanism of antimicrobial activity of lipids is not fully understood, there are a few possibilities to pursue. First, antimicrobial lipids may penetrate and disrupt the cell wall layer of bacteria. In a recent study, we observed that sphingolipids appeared to lyse *S. aureus*, but not *E. coli* (7). After incubation with sphingolipids, preparations of *S. aureus* contained lysed cells and identifiable fragments of the cell wall. Second, antimicrobial lipids may alter the cytoplasmic membrane of these bacteria. Bergsson et al. observed that fatty acids disrupted and disintegrated the

TABLE 3 Comparisons of the trapezoidal-AUC significance probabilities[a]

Bacterium	AUC of bacteria alone	Treatment lipids compared	AUC of treatment lipid	Trapezoidal-area significance probability (*P* value)[b]	
				Including controls	Excluding controls
E. coli	249.08	Phytosphingosine	3.82	0.0036[c]	0.10
		Sphingosine	0.75		
F. nucleatum	108.13	Phytosphingosine	1.67	0.000065[c]	0.0036[c]
		Sphingosine	1.00		
		Sapienic Acid	16.74		
S. aureus	201.04	Phytosphingosine	27.17	0.000065[c]	0.0036[c]
		Sphingosine	1.53		
		Lauric Acid	169.59		
S. sanguinis	180.98	Phytosphingosine	0.63	0.00052[c]	0.0214[c]
		Sphingosine	0.45		
		Sapienic Acid	0.40		
S. mitis	210.11	Phytosphingosine	2.09	0.000065[c]	0.0036[c]
		Sphingosine	14.79		
		Lauric Acid	46.43		
C. striatum	13.59	Phytosphingosine	5.92	0.000065[c]	0.0036[c]
		Sphingosine	8.07		
		Lauric Acid	0.78		

[a] AUCs for each bacterial species were compared across lipid treatments as a summary measure of viability over the time course. The significance probabilities of these comparisons are shown.
[b] Significance probability associated with the exact nonparametric Kruskal-Wallis test of the null hypothesis that the distribution of the trapezoidal area is the same across all treatment groups within a specified bacterial species.
[c] Significance at the 0.05 level.

cytoplasmic membrane of *C. albicans* (2). We also observed that sphingolipids appeared to alter the cytoplasmic membrane of *S. aureus*, but not *E. coli* (7). Third, it is also possible that antimicrobial lipids may directly penetrate the cell walls and cytoplasmic membranes of bacteria, enter, and disrupt the cytoplasm, similar to what was described by Bergsson et al. for *S. aureus* (3).

The extent to which microorganisms can metabolize sphingoid bases and fatty acids is not well known. It is possible that concentrations of lipids below the MIC can be tolerated and metabolized and concentrations of lipids above the MIC cannot. It is also possible that the bacteria used in this study can transport these lipids into the cell, accumulating them as intracellular inclusions. We recently observed that sphingolipids induced the formation of intracytoplasmic inclusions (7). Whether these inclusions are composed of accumulated lipids or bacterium-derived proteins is not yet known and is under investigation.

The high antimicrobial activity and low toxicity of lipids suggest that they may have applications as therapies to prevent or treat a wide variety of skin infections. These lipids are easy to obtain, have potent antimicrobial activities, and likely have low toxicity. In addition to direct antibacterial action, antimicrobial peptides are also chemotactic and can attract leukocytes to sites of infection (9, 12). The sphingoid bases are also inhibitors of protein kinase C and thus can modulate many biochemical actions. In addition, free sphingosine can be phosphorylated to produce sphingosine-1-phosphate, which is a potent bioactive metabolite that regulates diverse processes important for inflammation and immunity (21).

Phytosphingosine may be an ideal candidate for treating acne vulgaris (16, 18), as it has been shown to be antimicrobial for *Propionibacterium acnes in vitro*; downregulates the proinflammatory chemokines interleukin 8 (IL-8), CXCL2, and endothelin 1 in primary human keratinocytes; reduces the release of both lactate dehydrogenase and interleukin 1α in response to sodium dodecyl sulfate; is anti-inflammatory when tested in an organotypic skin model; and enhances the resolution of acne when applied topically. Lauric acid ($C_{12:0}$) has promise as a potential therapeutic for the treatment of acne (17). Lauric acid has MICs over 15 times lower than those of benzoyl peroxide and is not cytotoxic *in vitro* to human sebocytes or *in vivo* in mouse dermis.

Lipids common to the skin and oral cavity, D-sphingosine, phytosphingosine, dihydrosphingosine, sapienic acid, and lauric acid, had variable antimicrobial activities for a variety of Gram-positive and Gram-negative bacteria. Fatty acids and sphingoid bases may contribute to defensive barrier functions of the skin and oral cavity and may have potential for prophylactic or therapeutic intervention in infection.

ACKNOWLEDGMENTS

We thank Bonny Olson and Alissa Villhauer for their help with the assays in this project.

This work was supported by funds from R01 DE018032 and R01 DEO14390 from the National Institute of Dental and Craniofacial Research, National Institutes of Health.

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