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THE ROLES OF CUTANEOUS LIPIDS IN HOST DEFENSE

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Abstract

Lauric acid (C12:0) and sapienic acid (C16:1Δ6) derived from human sebaceous triglycerides are potent antimicrobials found at the human skin surface. Long-chain bases (sphingosine, dihydrosphingosine and 6-hydroxysphingosine) are also potent and broad-acting antimicrobials normally present at the skin surface. These antimicrobials are generated through the action of ceramidases on ceramides from the stratum corneum. These natural antimicrobials are thought to be part of the innate immune system of the skin. Exogenously providing these lipids to the skin may provide a new therapeutic option, or could potentially provide prophylaxis in people at risk of infection.

Keywords

long-chain base; sphingosine; dihydrosphingosine; phytosphingosine; lauric acid; sapienic acid; antimicrobial; bacteria

1. INTRODUCTION

The epidermis differentiates to produce a stratum corneum which provides a physically tough and relatively impermeable barrier for the skin (discussed by Feingold & Elias, this issue). This barrier not only limits water loss through the skin, which is essential for life on dry land, it limits the penetration of environmental substances that may reach the skin surface, including bacterial enzymes and toxins.

Through a variety of metabolic processes, the skin surface is mildly acidic. This factor limits the types of microorganisms that may grow there. In addition to this environmental factor, there is limited phosphate available to support microbial growth.

The lipids that underlie the permeability barrier provided by stratum corneum consist mainly of ceramides, cholesterol and saturated fatty acids containing 20 or more carbons. Saturated fatty acids of 20 or more carbons do not have antibacterial activity [1]. To the extent that we have tested them neither do cholesterol or ceramides; however, certain of the ceramides can be acted upon by ceramidases to release free long-chain bases, which are very potent and

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broad acting antibacterial agents. The release of sphingosine, dihydrosphingosine and 6-hydroxysphingosine occurs in the stratum corneum [2-5].

The skin surface is also coated with secreted sebaceous lipids. It has sometimes been suggested that sebum serves no biological function. Perhaps it once provided water-proofing, but since we no longer live in a wet environment this function is no longer necessary. However, it has become increasingly clear that sebum serves to deliver vitamin E to the skin surface, which inhibits oxidative damage to the skin. Also, several of the fatty acids that are released from sebaceous triglycerides are potent, though somewhat selective, antimicrobials. Lauric acid (C12:0) has long been recognized as one of the most potent antimicrobials out of a range of fatty acids or fatty acid derivatives [6]. It is a minor, often overlooked, but potentially biologically significant component of sebum. The most abundant fatty acid in human sebum is sapienic acid (C16:1Δ6), which is also a potent antibacterial agent [7]. There are also shorter fatty acids (C7-C11) which are almost always ignored, but which provide protection against certain fungi [8].

Several investigations done in the 1930s, most notably that of Burtenshaw [9], demonstrated that the skin had self-disinfecting power. In general, aqueous suspensions of strains of *Streptococcus pyogenes* or *Staphylococcus aureus* were applied to the skin surface, dried, and after some time the skin surface was scraped into a receiving medium. The total number of recovered bacteria was determined, and the number of viable cells was determined by allowing cells to grow on agar plates. The antibacterial nature of the skin surface was unequivocally demonstrated. However, it was suggested that a portion of this activity may have been due to the drying process, while low pH attributed to sweat was considered to be the most important factor.

While stratum corneum integrity, dry surface, low pH, antimicrobial peptides and products of endogenous nonpathogenic bacteria all contribute to the inhospitable environment for microbial growth presented by the skin surface, it is currently understood that certain lipids may be major contributors to this situation [6, 10, 11]. This review will summarize the literature on the antimicrobial lipids present at the skin surface.

2. FATTY ACIDS

The story of antimicrobial fatty acids at the skin surface begins with a 1942 study by Burtenshaw [12]. Burtenshaw tested the effects of saline and ether extracts of the skin surface as well as extracts of hair, nails and cerumen against various strains of *S. pyogenes*, *S. aureus* and several other bacteria in suspension culture. In general, the ether extracts were antimicrobial, and the saline extracts were inactive. This clearly suggested that the antimicrobial agent from the skin surface was lipoidal in nature. Although the complete chemical composition of human sebum was not known in 1942, it was known that both long and short-chain fatty acids and esters were present. Accordingly, it was speculated that fatty acids and/or their salts (soaps) due to their surfactant properties, may be responsible for the observed activity. Individual organic acids [acetic (C2:0), propionic caproic (C6:0) + Caprylic (C8:0), citric, ascorbic and uric acids] known to be present in human sweat and total fatty acids derived from butter [butyric (C4:0) + caproic (C6:0) + caprylic (C8:0) + capric (C10:0) + lauric acid (C12:0) + myristic acid (C14:0) + palmitic acid (C16:0) + stearic acid (C18:0ω9) + oleic acid (C18:1) + linoleic acid (C18:2ω6)] were tested for antibacterial activity. Capric acid, lauric acid, stearic acid and oleic acid were also tested individually. None of the organic acids from sweat had any activity. The total butter fatty acids as well as capric, lauric, stearic, oleic and linoleic acids were active. No ranking of relative potency of the active fatty acids or their salts was attempted. We now know that capric, lauric, myristic,

palmitic, stearic and small proportion of oleic acid and linoleic acid are present at the skin surface [13-15].

[In Burtenshaw's 1942 publication cited above, he acknowledges "Professor Alexander Flemming for generous facilities in the Inoculation Department of St Mary's Hospital". The first patient was treated with penicillin in 1942. By the time of the Normandy invasion in 1944, the military had a large supply of penicillin for use by the Allied troops. Only after World War II did penicillin become available for civilian use. Flemming shared a Nobel Prize in 1945.]

The earliest successful analysis of free fatty acids from human sebum was that published in 1947 by Weitkamp, Smiljanic and Rothman [13]. These investigators collected 45 kg of hair clippings from barber shops. They extracted the lipids into ethyl ether. The free fatty acids were recovered as potassium salts by shaking the ether extract with aqueous potassium hydroxide. The fatty acid salts were converted to methyl esters by treatment with acidified methanol. The fatty acid methyl esters were fractionated by fractional distillation under reduced pressure, which resulted in fractions containing from 7 through 22 carbons (16 fractions). Each fraction contained saturated fatty acids, and some fractions also contained monounsaturated entities. The C18 fraction was more complex and proved to contain additional species with two or more double bonds. The saturated and monoenic species were separated by crystallization of the saturated fatty acids. The most abundant fatty acid was C16:1. The double bond in this entity was oxidized to yield a dibasic fatty acid from the carboxylate end of the molecule and a monobasic acid from the methyl end of the molecule. The methyl ester of the monobasic acid was reanalyzed and the result proved that the C16:1 was C16:1 Δ 6, an unusual isomer different from the usual palmitoleic acid (C16:1 Δ 9). In fact, the name sapienic acid has been proposed for this fatty acid because it seems to be uniquely abundant in the sebum from *Homo sapiens*.

The main impetus for studying the fatty acid composition of human sebum was the observation that *tinea capitis*, a frequent recurring fungal infection of the scalp in infants and young children, was spontaneously cured at the onset of puberty [16, 17]. It was reasoned that since sebum secretion becomes significant at the onset of puberty, some component of sebum may be responsible for this spontaneous cure. When the fatty acids isolated in the above cited investigation were tested *in vitro* against *Microsporon audouinii*, the causative agent of ringworm of the scalp, the shorter odd-carbon, saturated fatty acids proved to be the antifungal agents. A related fatty acid from sweat, undecylenic acid, is used in numerous over the counter antifungal products today [17]. The earliest gas-liquid chromatographic analyses of sebaceous fatty acids confirmed that these short fatty acids are in fact of sebaceous origin [14, 15]. As noted, the short fatty acids from human sebum have been ignored in most subsequent analyses because of their relatively low abundance and because once boron trichloride/methanol and boron trifluoride/methanol came into wide use for preparation of fatty acid methyl esters, these shorter species were lost during sample preparation. This is unfortunate given the functionality of these short fatty acids.

More recently, lauric acid and sapienic acid have been screened for activity against a battery of common bacteria [18]. In general, both fatty acids were effective against Gram-positive bacteria (*S. aureus*, *Streptococcus sanguis*, *Streptococcus mitis*,) but not Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*); however, there were exceptions. Both lauric acid and sapienic acid were active against the Gram-negative *Fusobacterium nucleatum*, a periodontal pathogen [19]. Lauric acid was effective against the Gram-positive coryneform bacteria (*Corynebacterium bovis*, *Corynebacterium striatum*, *Corynebacterium jeikeium*), while sapienic acid was ineffective. Otherwise, when both fatty acids were active against a given bacterium, the potencies, judged by minimum inhibitory

concentrations or minimum bactericidal concentrations, sometimes varied widely. The kinetics of bacterial killing also varied widely depending upon the lipid-bacterium combination.

3. LONG-CHAIN SPHINGOID BASES

Sphingosine was discovered and named in 1884 by the German physician/chemist JLW Thudichum [20]. The name was intended to imply a sense of mystery, as in the sphinx. The earliest indication that sphingosine had antimicrobial properties came from a study done in 1948 in which the goal was to enhance the growth of tubercle bacilli in culture [21]. It was found that sphingomyelin increased the density of growth for a given incubation time and facilitated the initiation of growth from small inocula. Free sphingosine, however, inhibited growth. This potentially important finding was not followed up. However, relatively recent reports have shown that several synthetic dihydrosphingosine analogues are active against different strains of *Mycobacterium tuberculosis* in culture [22]. Sphingosine-1-phosphate has also been shown to induce antimicrobial activity against *M. tuberculosis* and *Mycobacterium smegmatis* both *in vitro* and *in vivo* through interaction with macrophages [23].

In 1989, Bibel et al. reported that lipids extracted from mouse stratum corneum had antimicrobial activity against a variety of skin-associated bacteria and fungi [24]. Furthermore, the lipids from essential fatty acid deficient mouse stratum corneum had greater activity than those from normal control mice. Several lipid fractions were found to be active, but no individual lipid was identified as being responsible for activity. Since one of the active fractions consisted of glycosphingolipids from which long-chain bases could be derived, several subsequent investigations focused on sphingosine and related molecular species [25]. Sphingosines and stearylamine were found to be effective against *S. aureus*. Sphingosine (D or D,L mixed isomers) were also effective against *Streptococcus pyogenes*, *Micrococcus luteus*, *Propionibacterium acnes*, *Brevibacterium epidermidis* and *Candida albicans* [25]. Subsequent work suggested that sphingosine interfered with cell wall synthesis following sphingosine interaction with cell membrane [26].

In more recent studies, sphingosine, dihydrosphingosine and phytosphingosine were shown to be similarly active against a range of Gram-positive and Gram-negative bacteria including *E. coli*, *F. nucleatum*, *S. aureus*, *S. sanguis*, *S. mitis*, *C. bovis*, *C. striatum* and *C. jeikeium* [27]. Minimum bactericidal concentrations were in the range of 0.3 – 63 micrograms per ml. As with the fatty acids, the kill kinetics were highly variable and depended upon the bacterium-base combination. None of the long long-chain bases was effective against *Pseudomonas aeruginosa* or *Serratia marcescens* [18]. It was shown that *S. aureus* and *E. coli* rapidly take up large amounts of long-chain base from culture medium, and this results in morphological changes [27].

4. INTERACTIONS

In addition to the antimicrobial lipids at the skin surface, there are also antimicrobial peptides and proteins [28, 29]. Most notable among these are the β -defensins, cathelicidins, psoriasin and RNases [28-31]. Preliminary results indicate that the antimicrobial peptides and antimicrobial lipids act synergistically [7, 32]. Specifically, sphingosine, dihydrosphingosine and phytosphingosine were examined in combination with the cathelicidin, LL-37, against a range of Gram-positive bacteria, Gram-negative bacteria and yeast. In general, there was synergy in killing the examined microorganisms. More work is needed in this area.

Synergy has also been noted between antimicrobial lipids and several exogenous antimicrobials. This includes synergy between low concentrations of ethanol and sapienic acid to achieve rapid and complete killing of *S. aureus* that was resistant to both methicillin-resistant and mupirocin-resistant [7]. Synergy has also been seen between chlorhexidine and lauric acid in killing *Streptococcus mutans* [33]. N-Lauryl-arginine ethyl ester laurate synergized with phytosphingosine in killing of *Corynebacterium bovis* and *Coryneform striatum* [34].

In another study, phytosphingosine was shown to enhance the response to benzoyl peroxide of acne lesions [35]. It was not clear that this interaction was synergistic since there was no phytosphingosine only treatment group. Interestingly, phytosphingosine alone was shown to be antimicrobial against *Propionobacterium acnes* and anti-inflammatory.

Interestingly, antimicrobial lipid production in epidermis increases when permeability barrier function is artificially impaired, but this response can be blocked by provision of an artificial barrier [36, 37]. It appears that permeability barrier and antimicrobial barrier functions are coordinately regulated. In accord with these findings, it has been found in human subjects with atopic dermatitis, who have compromised permeability barrier function, there is elevated β -defensin [38]. These same subjects have lower than normal levels of both sapienic acid and free sphingosine, which is thought to explain their greater susceptibility to colonization by *Staphylococcus aureus* [39, 40].

5. SUMMARY

Lauric acid (C12:0) and sapienic acid (C16:1 Δ 6) derived from human sebaceous triglycerides are potent antimicrobials found at the human skin surface. Long-chain bases (sphingosine, dihydrosphingosine and 6-hydroxysphingosine) are also potent and broad-acting antimicrobials normally present at the skin surface. These antimicrobials are generated through the action of ceramidases on ceramides from the stratum corneum. These natural antimicrobials are thought to be part of the innate immune system of the skin. Exogenously providing these lipids to the skin may provide a new therapeutic option, or could potentially provide prophylaxis in people at risk of infection.

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Highlights

- Sphingoid bases, and certain fatty acids are antimicrobials at the skin surface.
- Long-chain bases are derived from ceramides through the action of ceramidases.
- The antimicrobial fatty acids are derived from sebaceous triglycerides.
- The activities of antimicrobial lipids vary with the microbe.