Acta Derm Venereol 2015; 95: 783–786

INVESTIGATIVE REPORT

Reduced Expression of Dermcidin, a Peptide Active Against *Propionibacterium acnes*, in Sweat of Patients with Acne Vulgaris

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Dermcidin (DCD), an antimicrobial peptide with a broad spectrum of activity against bacteria such as *Propionibacterum acnes*, is expressed constitutively in sweat in the absence of stimulation due to injury or inflammation. The aim of this study was to determine the relationship between DCD expression and acne vulgaris associated with *P. acnes*. The antimicrobial activity of recombinant full-length DCD (50 μg/ml) was 97% against *Escherichia coli* and 100% against *Staphylococcus aureus*. Antimicrobial activity against *P. acnes* ranged from 68% at 50 μg/ml DCD to 83% at 270 μg/ml DCD. DCD concentration in sweat from patients with acne vulgaris (median 9.8 μg/ml, range 6.9–95.3 μg/ml) was significantly lower than in healthy subjects (median 136.7 μg/ml, range 45.4–201.6 μg/ml) ($p = 0.001$). DCD demonstrated concentration-dependent, but partial, microbicidal activity against *P. acnes*. These results suggest that reduced DCD concentration in sweat in patients with inflammatory acne may permit proliferation of *P. acnes* in pilosebaceous units, resulting in progression of inflammatory acne. *Key words: dermcidin;* Propionibacterum acnes*; antimicrobial peptide; sweat.*

Accepted Feb 11, 2015; Epub ahead of print Feb 12, 2015

Acta Derm Venereol 2015; 95: 783–786.

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Acne vulgaris, a chronic inflammatory lesion of pilosebaceous units, often develops on the face, anterior chest and upper back of adolescents (1). It is a common skin disease, affecting 35 to over 90% of those in adolescence (2, 3). Acne lesions are typically classified as non-inflammatory (open and closed comedones) or inflammatory (papules and pustules). Seborrhoea (or grease production) is also a feature of acne (4). Inflammatory acne in particular is difficult to treat, often increases in severity, and may cause scarring that can have a negative effect on the patient (5, 6).

The bacterium *Propionibacterium acnes*, a major inhabitant of human hair follicles and sebaceous glands, plays a central role in the pathogenesis of acne vulgaris (7, 8). In comedogenesis in acne vulgaris, pathogenic factors include the release of free fatty acids from triglycerides in sebum (9, 10) due to lipase produced by *P. acnes*, inflammation triggered by the oxidation of squalene induced by porphyrin from *P. acnes* (11), and increased sebum production due to the activation of diacylglycerol acyltransferase in sebaceous cells (12, 13). In the inflammatory lesion, *P. acnes* colonization occurs in the anaerobic environment created by the formation of closed comedones (to form a papular lesion) (14). *P. acnes* appears to intensify the inflammation via extracellular enzymes (15), antigenicity (2, 16, 17), chemoattractant (18), and complement activation (19, 20), as well as inflammation induction through toll-like receptors 2 and 4 in keratinocytes (21) and inflammation activation (22). Thus, *P. acnes* is involved in the development of inflammatory acne. However, biogenic factors that may control the colonization of *P. acnes* during the transition from the comedo stage to the inflammatory acne stage, have not yet been identified.

Cutaneous defence mechanisms include a physical barrier, a biological barrier, and anti-bacterial substances (23).

Antimicrobial substances, both lipid and proteinaceous, are secreted from the sebaceous glands. Among lipid antimicrobial substances, palmitoleic acid, an unsaturated fatty acid, has a strong antimicrobial activity against gram-positive bacteria (24). Antimicrobial peptides, which are proteinaceous antimicrobial substances, are important defence factors in innate immunity. Psoriasin, cathelicidin, β-defensin and dermcidin (DCD) are well-characterized antimicrobial peptides present in human dermis.

DCD, an antimicrobial peptide of 110 residues with a molecular weight of 9.3 kDa, is specifically expressed in sweat glands and, in the absence of an inflammatory stimulus, is constitutively secreted from eccrine glands as full-length DCD (25). Furthermore, DCD, which is secreted with sweat and enzymatically broken down, covers the skin surface in several degraded forms. It is possible that DCD affects colonization and growth of resident flora. Sweat mixes with sebum at the openings of pores, where amphipathic DCD and *P. acnes* may come into contact with each other. The aim of this study was to elucidate the relationship between acne vulgaris and DCD, by preparing a recombinant DCD (rDCD)

 doi: 10.2340/00015555-2068
 ISSN 0001-5555

peptide, studying the antimicrobial activity of DCD against *P. acnes*, which is a major factor in the onset of acne vulgaris, and investigating the DCD concentration in the sweat of patients with acne vulgaris.

MATERIALS AND METHODS (see Appendix S1[1])

RESULTS

Expression and purification of rDCD

pTKK19-ubinew-DCD plasmid (Mitsubishi Chemical Institute of Life Sciences, Tokyo, Japan) was introduced into *E. coli* BL21 (DE3) strain where recombinant histidine (rHis) ubiquitin-DCD was expressed as a fusion protein. Purified rDCD had an apparent molecular weight of ~16 kDa on SDS-PAGE (15% acrylamide, 0.3% bisacrylamide [w/v]) (Fig. 1, Lane 2). However, the molecular weight, as determined by MALDI-TOF MS, was 9,253 Da (data not shown). The protein that migrated to ~16 kDa, as assayed by Western blotting, reacted to a mouse anti-DCD monoclonal antibody (mAb) 10C3, which had been prepared using sweat as the source of immunity (Fig. 1, Lane 4). This confirmed that the rDCD had been purified.

Antimicrobial activity against E. coli *and* S. aureus

To confirm that rDCD had been purified as an active form, antimicrobial activity against *E. coli* and *S. aureus* was studied. Antimicrobial activity of an active form of DCD against *E. coli* and *S. aureus* has been reported by Schittek et al. (25). The concentration of rDCD was determined using the mean DCD concentration of 38 subjects obtained from 18–45-year-old healthy Japanese general population without acne vulgaris (mean 50 μg/ml; data not shown) as a reference. According to the study, the killing rate of rDCD against *E. coli* was 97%, and the rate against *S. aureus* was 100% (Fig. 2A).

Antimicrobial activity against P. acnes

Once the purification of rDCD, which has an antimicrobial activity against *E. coli* and *S. aureus*, was confirmed, its antimicrobial activity against *P. acnes* was studied. The study revealed that the antimicrobial activity against *P. acnes* was 68% with 50 μg/ml rDCD, and 83% with 270 μg/ml rDCD (Fig. 2B). The antimicrobial activity of DCD against *P. acnes* was concentration dependent.

Protein and DCD in sweat of patients with acne vulgaris

Based on the hypothesis that DCD in sweat keeps the growth of *P. acnes* in the skin under control and is involved in inflammatory acne development, DCD concentrations in sweat were compared between patients with inflammatory acne and healthy subjects. Sweat samples were collected from 15 patients with acne vulgaris (median age 20 years, 18–39-year-olds, male/female ratio 7:8). The healthy control group had 14 people with no history of inflammatory acne (median age 22 years, 18–34-year-olds, male/female ratio 8:6) (Table SI[1]). The distribution of total protein concentration in the sweat of patients with acne vulgaris was in the range 56–1,329 μg/ml, where 87% were in the range 100–1,000 μg/ml. In the healthy subject group, the distribution was in the range 186–2,310 μg/ml, where 86%

[1]https://doi.org/10.2340/00015555-2068

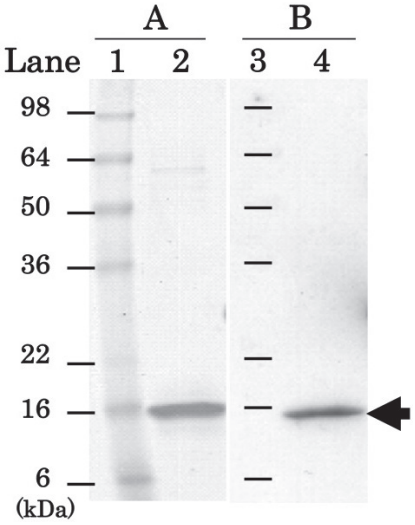

Fig. 1. Purified recombinant dermcidin (rDCD). (A) Sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE). (B) Western blot analysis of purified rDCD using a mouse anti-DCD 10C3 monoclonal antibody (mAb). *Lanes 1 and 3:* molecular weight markers. *Lanes 2 and 4:* rDCD. The molecular weights of these recombinant proteins, analysed by Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS), were equal to the theoretical value from the amino acid sequence (9.3 kDa).

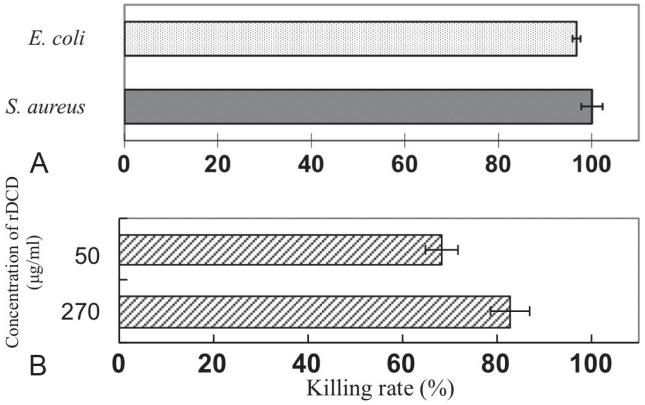

Fig. 2. Antimicrobial activities against; (A) *E. coli* and *S. aureus* and (B) *P. acnes.* The antimicrobial effect of recombinant dermcidin (rDCD) against *E. coli*, *S. aureus* and *P. acnes* was evaluated by colony-forming unit assay. The concentration of rDCD used in *E. coli* and *S. aureus* assays was approximately 50 μg/ml. *Error bar:* standard error.

were in the range 100–1,000 μg/ml. These data suggest that the distribution of the total protein concentration in sweat is approximately the same in patients with acne vulgaris and healthy subjects. However, DCD concentrations in patients with acne vulgaris were in the range 4.2–167 μg/ml, and that of healthy subjects were in the range 31.9–342 μg/ml (Fig. 3). The median concentration of DCD peptide in the sweat of patients with acne vulgaris was significantly lower (median 9.8 μg/ml, interquartile range 6.9–95.3 μg/ml) than in the healthy control group (136.7 μg/ml, 45.4–201.6 μg/ml) ($p = 0.001$) (Fig. 4).

DISCUSSION

P. acnes, thrives in weakly acidic conditions and helps to maintaining the surface of the horny layer weakly acidic; thus, growth of other pathological microbes is suppressed, and their settling is prevented (23). On the other hand, after comedo closing, excessive proliferation of *P. acnes* relates to the exacerbation of acne vulgaris. Peptidoglycan, an element of *P. acnes* cell wall, induces inflammatory cytokines such as interleukin (IL)-6 and IL-8 via toll-like receptors 2. Migration and invasion by neutrophils and macrophages are also induced. Based on these findings, proliferation of *P. acnes* is considered as the primary cause of inflammation in acne vulgaris (28).

The process of controlling foreign and resident microbes in the horny layer appears to involve palmitoleic acid and sphingosine (a ceramide metabolite), categorized as lipid antimicrobial substances, and psoriasin, cathelicidin, β-defensin, and DCD, categorized as protein antimicrobial substances. Palmitoleic acid, which constitutes an intercellular lipid of the horny layer, has antimicrobial activity against *S. aureus*, *Streptococcus salivarius* and *Fusobacterium nucleatum* (24). Sphigosine exhibits a broad spectrum of antimicrobial activity against microbes, such as *Streptococcus pyogenes*, *Micrococcus luteus*, *P. acnes* and *Candida albicans* (29). A reduction in sphigosine may cause a decline in the antimicrobial activity of the horny layer and contribute to the settling of *S. aureus*, but whether sphigosine actually demonstrates antimicrobial activity *in vivo* is unknown. Proteinaceous antimicrobial substances, such as psoriasin, cathelicidin and β-defensin are produced by keratinocytes in response to inflammatory irritation. Psoriasin reacts with gram-negative bacteria, cathelicidin reacts with gram-positive bacteria, gram-negative bacteria, and some fungus. β-Defensin-2 reacts with gram-positive bacteria and some fungus. It is reported that resident bacterial flora of human skin, including *P. acnes*, has resistance to proteinaceous antimicrobial substances (30). DCD, a peptide first reported by Schittek et al. (25) in 2001, is expressed specifically as a full-length DCD, in sweat glands, and is constitutively secreted from eccrine glands in the absence of an inflammatory stimulus. DCD has 14 cleavage fragments, and its antimicrobial activity differs depending on the cleavage. From studies conducted so far on synthetic DCD and rDCD, it is now clear that DCD at concentrations of 10–100 μg/ml demonstrates microcidal activity against *S. aureus*, *E. coli* and *Enterococcus faecalis*, and, at 50–100 μg/ml, against *C. albicans* (25). DCD also exhibits antimicrobial activity against *Pseudomonas sputita* (30) as well as *Salmonella typhimurium* and *Listeria monocytogenes* (31).

We determined that the antibacterial activity of DCD against *P. acnes* increases in a concentration-dependent

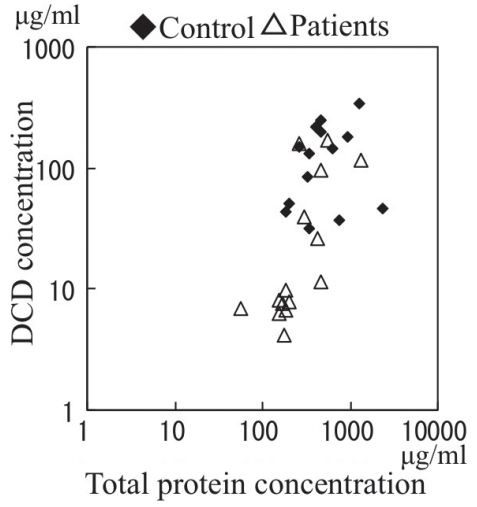

Fig. 3. Quantitative analysis of dermcidin (DCD) peptides in sweat samples. Each individual value of DCD concentration and total protein concentration in sweat samples in healthy control (*solid diamond*) and patients with acne vulgaris (*white triangle*).

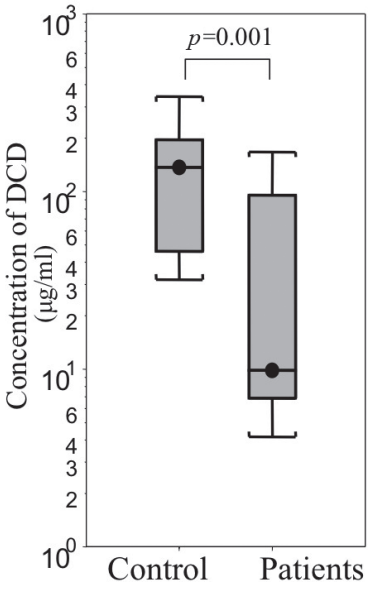

Fig. 4. Means of concentration of dermcidin (DCD) peptides in sweat samples between healthy control and patients with acne vulgaris. *Error bar:* standard error.

manner. Analysis of DCD concentration in sweat of inflammatory acne patients revealed that the DCD concentration in the inflammatory acne patients was significantly lower than that in healthy individuals. This finding suggests that inflammatory acne patients may have a reduced control of the *P. acnes* population.

Rieg et al. (32) studied DCD concentrations in the sweat of patients with atopic skin inflammation, and suggested the correlation between the decline in DCD concentration, and the settling of *S. aureus* and susceptibility to skin infection such as impetigo contagiosa. Our study suggests that in patients with inflammatory acne, reduced DCD concentration in sweat permits the proliferation of *P. acnes* in pilosebaceous units, and leads to the progression of inflammatory acne. Further research into total DCD concentration and DCD fragments, based on this study, will improve our understanding of the relationship between DCD and acne vulgaris and may lead to the development of effective prevention of inflammatory acne using active rDCD.

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