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Synergistic effect of antibacterial agents human β-defensins, cathelicidin LL-37 and lysozyme against *Staphylococcus aureus* and *Escherichia coli*

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Human β-defensin;
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Summary

Background: The antimicrobial properties of the skin are attributed to several agents including human β-defensins (hBDs), cathelicidin LL-37 and skin lysozyme. Although these antibacterial agents reside in the skin to protect it against infection, it is not well known whether the total analysis of all combinations of these agents may result in synergistic effect to enhance their antibacterial activities against invading micro-organisms.
Objective: To elucidate the interactions between keratinocyte-derived antibacterial agents in the extracellular milieu, we investigated the individual and synergistic activities of hBDs, LL-37 and lysozyme against *Staphylococcus aureus* and *Escherichia coli* in neutral and acidic milieus.
Methods: The colorimetric method using alamarBlue was employed to assess the antibacterial activities of hBD-1, -2, -3, LL-37 and lysozyme and the viability of bacteria was read spectrophotometrically.

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Results: In both neutral and acidic pH milieus, hBD-1, -2, -3, LL-37 and lysozyme exhibited antibacterial activity against *S. aureus* and *E. coli* in a dose-dependent manner. Interestingly, the antibacterial activity of hBD-1, -2, -3 and lysozyme but not LL-37 was significantly enhanced in acidic milieu (pH 4.6). Furthermore, various combinations of above agents resulted in a synergistic or additive antibacterial effect against *S. aureus* and *E. coli* in neutral milieu. The synergistic effect of hBDs, LL-37 and lysozyme against *S. aureus* was further significantly enhanced in acidic milieu. In contrast, above antibacterial agents exhibited mainly additive rather than synergistic effect on antibacterial activity against *E. coli* in acidic milieu.
Conclusion: Taken together, these results provide a novel evidence of antimicrobial mechanism of natural human skin-derived antibacterial agents against bacterial infection, and their involvement in innate immunity.
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1. Introduction

The bactericidal and fungicidal properties of the skin tissues have been attributed to several agents including antimicrobial peptides and skin lysozyme. In humans, over a dozen of antibacterial peptides have been identified, and comprise histatins, granulysin, lactoferricin, α- and β-defensins and cathelicidin human cationic antibacterial protein of 18 kDa (hCAP18)-derived LL-37 [1]. As effectors of innate immunity, the antimicrobial agents including antibacterial peptides and lysozyme exhibit a broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi and certain viruses [1,2].

The most investigated human antibacterial peptides are human α- and β-defensins, and cathelicidin LL-37. The α- and β-defensins differ from one another by the spacing and the connectivity of their characteristic six cysteine residues. Additionally, α-defensins are found in neutrophils and Paneth cells of small intestinal, whereas β-defensins are products of epithelial tissues [1]. To date, six human β-defensins (hBD)-1 through 6 have been identified. hBD-1 is constitutively produced by various epithelial tissues including urogenital and respiratory tracts [3]. hBD-2 was originally isolated from extracts of lesional scales from psoriatic skin, and is mainly present in skin and respiratory as well as gastrointestinal tracts [4]. It is inducibly expressed in inflamed skin lesions upon treatment with bacterial lipopolysaccharide and cytokines, such as tumor necrosis factor-α and interleukin (IL)-1β [4—6]. In addition to its antibacterial activity, hBD-2 activates several cells, such as dendritic cells, mast cells and neutrophils [7—10]. hBD-3 was also isolated from human lesional psoriatic scales, and is expressed in epithelial as well as non-epithelial tissues including heart, liver and skeletal muscle [11]. As for hBD-4, it is up-regulated by infection with Gram-positive and Gram-negative bacteria in human respiratory epithelial cells [12]. The very recently discovered hBD-5 and hBD-6 are specifically expressed in human epididymis; however, their antimicrobial activities are not yet well known [13].

Another family of antibacterial peptides named cathelicidins has been identified in myeloid cells and epithelial tissues of humans and animals. The in vivo importance of cathelicidins in skin host defense has been demonstrated in mice null of the expression of CRAMP, a homolog of human cathelicidin LL-37, that were more susceptible to skin infections caused by group A *Streptococcus* than wild-type mice were [14]. Although about 20 cathelicidin members are present in mammalians, only one cathelicidin, hCAP18 has been found in humans thus far, and its mature antibacterial peptide LL-37 is mainly expressed in neutrophils and epithelium [15—17]. The expression of LL-37 is up-regulated in keratinocytes during inflammation. Besides its bactericidal activity, LL-37 can bind to lipopolysaccharide and blunt some of its biological effects, and activates the migration of monocytes, neutrophils and mast cells [18—20].

Lysozyme, also referred to the trivial name muramidase, is ubiquitously present in various human tissues and secretions, and exhibits antimicrobial activities against different microorganisms. Ogawa, one of the authors, is the first researcher to isolate and characterize human skin lysozyme [21]. He has found that the content of lysozyme was 60—120 μg per wet g of human skin [21—23]. The bacterial killing mechanism of lysozyme consists in the lytic and non-lytic mechanisms. The lytic mechanism includes enzymic peptidoglycan hydrolysis (muramidase activity) where lysozyme provokes cell lysis by hydrolyzing the peptidoglycan layers of bacteria, and induction of autolysins that are capable of causing bacterial autolysis [2,24]. The non-lytic mechanism is principally based on the properties of lysozyme to cause membrane perturbation of its targets through the binding of a certain domain of

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