Comparison of Efficacy of Doxycycline and Isotretinoin on Cutaneous Human Beta-Defensin-1 and -2 Levels in Acne Vulgaris

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Abstract
Background: Recent studies have shown that human beta-defensin-1 (hBD-1) and human beta-defensin-2 (hBD-2), which are antimicrobial peptides produced by the skin, play a role in the pathogenesis of acne vulgaris (AV). Objective: The aim of this study was to determine the role of antimicrobial peptides in the pathogenesis of AV and enlighten the effects of doxycycline and isotretinoin in the expression of these defensins in AV. Materials and Methods: A total of 44 patients (22 patients in each group) with Grade 6 and 8 AV who were indicated doxycycline or isotretinoin for their treatment, and 20 healthy volunteers were included in this study. Pretreatment cutaneous samples were obtained from pustular lesions and uninvolved skin of AV patients and were repeated after the treatment. Only one biopsy was obtained from controls. Results: Cutaneous levels of hBD-1 and hBD-2 were significantly increased in AV patients when compared with healthy controls (P<0.05). Doxycycline therapy achieved a decrease in hBD-1 levels (P<0.05), whereas isotretinoin therapy achieved a reduction in hBD-2 levels when compared with pretreatment levels (P<0.05). Posttreatment hBD-1 and hBD-2 levels were not different between doxycycline and isotretinoin groups (P>0.05). Conclusion: In the light of these results, it was reasonable to assume the role of hBD-1 and hBD-2 in the pathogenesis of AV. Our results showing a significant reduction in hBD-1 staining with doxycycline treatment and in hBD-2 with isotretinoin suggested that some part of their anti-acne effect worked through these mechanisms.

Key Words: Acne, doxycycline, human beta-defensin, isotretinoin

Introduction
Acne vulgaris (AV) is a multifactorial disease of the pilosebaceous unit. Keratinocytes and sebocytes in pilosebaceous unit play a role in the identification of pathogens and are involved in immune responses through releasing antimicrobial peptides.⁴⁻⁷ Studies in recent years have shown that antimicrobial peptides, including cathelicidins, defensins, human neutrophil peptides, RNases, and S100 proteins, have important roles in the cutaneous innate immunity, and they have been reported to exert pro-inflammatory effects possibly through dysregulation of the immunomodulatory activities. Antimicrobial peptides may also play a beneficial role in AV by acting against Propionibacterium acnes.⁴⁻⁶ Defensins, a small antimicrobial peptide family, are cationic proteins which are expressed in all human epithelial tissues and are excreted as a response to microbial infections; include 28–42 amino acids and contain three disulfide bonds. They have two subtypes as alpha and beta. Alpha-defensins are located in neutrophil granules or intestinal Paneth cells while beta defensins are produced by various epitheliums. Human beta-defensin-1 (hBD-1) was first isolated from blood filtrates, while human beta-defensin-2 (hBD-2) was first isolated from psoriatic squam.⁹⁻¹⁴ In recent studies, the role of dysregulation of specific antimicrobial peptides, are stressed in acne development.³⁻⁸ The identification of elevated cathelicidin and hBD-1 levels in both inflammatory lesions and serum in the patients with AV when compared with controls possibly supports the role of antimicrobial peptides in the etiopathogenesis of AV.⁵⁻⁷ P. acnes whose

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colonization is increased in AV is suggested to increase hBD-1, and hBD-2 release beside chemokines from keratinocytes and mononuclear cells and thereby leading to inflammatory cells to accumulate at infection site.\(^{[15-19]}\) Tetracyclines are suggested to act through the inhibition of \(P.\) \textit{acnes} and also through the inhibition of production of \(P.\) \textit{acnes}-mediated inflammatory mediators.\(^{[15-19]}\) The recovery provided by tetracyclines in inflammatory and noninflammatory acne lesions even in subantimicrobial doses suggests that it could be effective also through other mechanisms.\(^{[19]}\) Isotretinoin is the only therapeutic agent which has an effect on all etiopathogenetic mechanisms in acne pathogenesis; however, it is not clearly understood through which mechanisms the effect occurs.\(^{[17,18,20]}\)

In our study, we aimed to enlighten the role of antimicrobial peptides in the pathogenesis of AV and document the effects of doxycycline and isotretinoin treatments on the levels of cutaneous hBD-1 and hBD-2 to understand the potential mechanisms of their anti-acne, particularly anti-inflammatory effects.

**Materials and Methods**

**Sample selection**

A total of 44 patients aged between 18 and 35 years (22 patients in each group) who attended the acne polyclinic of the Department of Dermatology, Gazi University between November 2010 and March 2011, who were diagnosed with stage 6–8 AV according to Allen-Smith scale and who had the indications for systemic doxycycline or isotretinoin use were included in the study. About 20 healthy volunteers who did not have AV or any other inflammatory skin disease, matched with patients group with regard to mean age and gender were taken as control group. Local Ethics Committee approval and written informed consent were obtained before the study. Exclusion criteria were as follows: patients receiving topical or oral antibiotics or any other anti-acne medications during the 4 weeks before the study; lactating patients or those with known or suspicion of pregnancy; the patients with hyperlipidemia, cardiac diseases or any other systemic or cutaneous diseases; and those with a history of hypersensitivity to isotretinoin or tetracyclines.

The isotretinoin treatment (0.5[mild]–1[severe] mg/kg/day) was continued until cumulative dose reached to 120–150 mg/kg. Doxycycline (100 mg/day) was continued for 3 months depending on the clinical response. Liver function tests (both treatment groups), renal function tests (doxycycline group), and fasting lipids (isotretinoin group) were evaluated at the baseline, and afterward monthly during the treatment. The patients were clinically examined every month until the complete clearance of all lesions. The effectiveness of treatment was evaluated according to Allen-Smith scale at the end of treatment as follows: full recovery, no lesion or Stage 0–2 acne lesions; partial recovery, Stage 4 acne lesions, and no recovery, no significant recovery.

At the beginning of the study, two punch (3 mm) skin biopsies in acne areas (back or shoulder region) were obtained from each patient, one from an established pustular lesion and one from uninvolved skin of the same patient. After the complete clearance of the lesions, repeat biopsies were taken from the identical region of the skin that was biopsied before the therapy. Only one biopsy from the upper back region was obtained from healthy controls. These specimens were reserved for immunohistochemical investigation.

**Assessment of biopsy samples**

Sections (4 \(\mu\)m) were cut from the specimens and were stained for hBD-1 and hBD-2 according to the protocol of the manufacturer. Staining was done using the streptavidin-biotin triple indirect immune-peroxidase method to determine hBD-1 and hBD-2 expressions in AV cases; hBD-1 (Lo145235, rabbit polyclonal, Abbiotec®) and hBD-2 (Lo145235, rabbit polyclonal, Abbiotec®) antibodies were used. Biotin (secondary) antibody, streptavidin-biotin complex, and 3-amino-9-ethylcarbazole used as chromogen were commercially available kits. Lymph node tissue was used as positive control of hBD-1 and hBD-2 antibodies. The immunohistochemical assessment was performed according to the cytoplasmic staining severity of cells stained with hBD-1 or hBD-2 in randomly selected, four consecutive epidermal fields. The staining severity was rated as negative (0), mild (+1), moderate (+2), and severe (+3) [Figure 1]. All histological slides were reviewed by the same pathologist, without knowledge of the patient groups.

**Statistical analysis**

Data were analyzed with SPSS Statistics for Windows, version 15.0 (SPSS Inc., Chicago, III., USA). Descriptive statistics (frequency, mean), Chi-square test, Yates correction Chi-square test were used. \(P<0.05\) was accepted as statistically significant.

**Results**

**Age, gender, and treatment responses**

No statistically significant difference was detected among isotretinoin, doxycycline, and control groups with regard to age (mean age: 20.91±3.06 years, 21.64±3.38 years, and 20.95±3.06 years, respectively) and gender (female/male: 15/7, 14/8, and 11/9, respectively) distribution (\(P>0.05\)).

In isotretinoin group, pretreatment acne severity was Stage 6 in 13 (59.1%) cases and Stage 8 in 9 (40.9%)
cases; in doxycycline group, acne severity was Stage 6 in 16 (72.7%) cases and Stage 8 in 6 (27.3%) cases. No significant difference between the groups was noticed regard to acne severity \( (P > 0.05) \). Both of the treatments, isotretinoin (50% had Grade 0, 45.5% had Grade 1, and 4.5% had Grade 2 acne) and doxycycline (18.2% Grade 0, 72.8% had Grade 1, and 9.1% had Grade 2 acne), achieved full recovery of skin lesions in all the patients. Isotretinoin and doxycycline were well tolerated by the patients, and treatment was discontinued in only one patient in isotretinoin group due to depressive symptoms. Some patients complained of mild cutaneous side effects like mucosal dryness, eczematous dermatitis, and cheilitis.

**Beta-defensin levels**

Table 1 shows the amount of immunohistochemical hBD-1 and hBD-2 staining of the sections in the skin of AV patients and controls. Statistical analysis showed more hBD-2 staining \( (P < 0.05) \), but not hBD-1 staining \( (P > 0.05) \), in the pustular lesions of AV patients, when it was compared with those of uninvolved skin.

![Figure 1: The immunoreactivity of human beta defensin-1 in the skin of acne patients (a and b) and cytoplasmic staining of human beta-defensin-2 in the skin of controls (c) (Avidin-biotin complex staining, ×400)](image)

**Table 1: The amount of human beta-defensin-1 and human beta-defensin-2 staining in acne vulgaris patients and in controls**

| Patient group | hBD-1, n (%) | hBD-2, n (%) |
|---------------|--------------|--------------|
|               | Severe       | Moderate     | Mild         | Negative    | Severe       | Moderate     | Mild         | Negative    |
| Isotretinoin  |              |              |              |             |              |              |              |             |
| Pre-T         | 10 (45.4)    | 5 (22.7)     | 3 (13.6)     | 4 (18.1)    |              |              |              |             |
| Post-T        | 4 (19)       | 2 (9.5)      | 8 (38.1)     | 7 (33.3)    |              |              |              |             |
| Doxycycline   |              |              |              |             |              |              |              |             |
| Pre-T         | 6 (27.3)     | 10 (45.4)    | 6 (27.3)     | -           |              |              |              |             |
| Post-T        | 1 (4.8)      | 6 (28.6)     | 10 (47.6)    | 4 (19)      |              |              |              |             |
| Uninvolved skin | 9 (20.5) | 17 (38.6) | 11 (25) | 7 (15.9) |              |              |              |             |
| Controls      | -            | 3 (15)       | 11 (55)      | 6 (30)      |              |              |              |             |

hBD-1: Human beta-defensin-1, hBD-2: Human beta-defensin-2, Pre-T: Pretreatment, Post-T: Posttreatment

Pretreatment hBD-1 and hBD-2 levels in the pustular lesions were found statistically significantly higher in the patient group compared to control group \( (P < 0.05) \) [Table 2].

While statistical analysis showed a significant decrease in hBD-1 levels after doxycycline treatment \( (P < 0.05) \) and in hBD-2 levels after isotretinoin treatment \( (P < 0.05) \), a difference was not observed between pretreatment and posttreatment hBD-1 staining levels in isotretinoin group and hBD-2 staining levels in doxycycline group \( (P > 0.05) \), each. When posttreatment stainings of hBD-1 and hBD-2 in isotretinoin and doxycycline groups were evaluated, no significant difference was detected between the two treatment groups nor between treated and control groups \( (P > 0.05) \) [Table 3].

**Discussion**

Our current study confirmed the observation that antimicrobial peptides, hBD-1 and hBD-2, which had antimicrobial activity and immunological properties, were expressed in pustular lesions of acne patients and were significantly higher when compared with that of the healthy controls. This finding, together with the existing data, supported the role of hBD-1 and hBD-2 in acne pathogenesis. The previous studies had shown hBD1 and hBD2 expression in distal of outer root sheath and pilosebaceous canal where the microbial invasion was intensive in pilosebaceous follicles of both healthy volunteers and AV lesions. Defensin expression was limited to suprabasal layers in pilosebaceous canal and interfollicular cells and was prominent in the center of outer root sheath and hair root. Considering the location of epidermal stem cells in outer root sheath center and hair root, hBD expression in this region might serve to protect these stem cells from microbial invasion.

In our study, we observed that hBD-2, but not hBD-1 levels, were higher in pustular lesions when compared with that of uninvolved skin of acne patients. In the study of Chronell et al., hBD-1 was found to be
expressed in papular lesions most, followed by comedone and pustule, hBD-2 expression was seen to be maximum in pustular lesions, followed by papule and comedone. This study which indicated that hBD-1 and hBD-2 might be in different levels in different elemental acne lesions might also highlight the difference detected in our hBD-1 and hBD-2 levels in pustular lesions. Doxycycline acts through reducing free fatty acids in sebum and extracellular lipase besides inhibiting P. acnes. Doxycycline is also known to show anti-inflammatory effect through inhibition of neutrophil chemotaxis and release of pro-inflammatory cytokines such as tumor necrosis factor alpha, interferon-gamma (IFN-γ), and MMP-9.[6,10,21-24] In our study, doxycycline treatment achieved statistically significant reduction of hBD-1 levels, but hBD-2 levels were similar when compared to pretreatment values. Therefore, we considered different factors to influence or regulate these two peptides. Human beta defensin-1 was known to be a peptide which was responsible for antimicrobial activity in basal layer of skin and was affected less from external stimuli.[14,23-25] Bacterial lipopolysaccharides and IFN-γ were shown to play major role for increasing hBD-1 release[23] while surgery in normal skin[25] and cyclooxygenase (COX-2) in keratinocyte cultures[14,24] were factors that were involved in hBD-2 release. Therefore, despite the fact that our data did not allow to draw any conclusion about the exact reason for posttreatment decrease in hBD-1 levels and unchanged hBD-2 levels might be related to role of doxycycline in inhibiting IFN-γ or to the interactions between COX-2,[23] and doxycycline. It had been observed that anti-inflammatory activities could occur in subantimicrobial doses where no antibacterial effect was available. This suggested that doxycycline provided therapeutic efficacy with different mechanisms other than antibacterial activity in AV treatment. Low doses of doxycycline might also suppress inflammation, possibly through different mechanisms such as the regulation of inflammatory cytokines or the regulation of antimicrobial peptides.[15-19] Another study with subantimicrobial doses of doxycycline might provide a clearer picture of the antiinflammatory effect of doxycycline through modification of defensin synthesis.

Isotretinoin is an effective drug used in acne treatment as it is effective in all major mechanisms which are responsible for acne pathogenesis. Isotretinoin leads to sebaceous gland atrophy through inhibiting basal sebocyte proliferation, and decreases the release of pro-inflammatory mediators through reduction of neutrophil migration and P. Acnes population.[17,18,27] Because beta-defensin release was influenced by pro-inflammatory cytokines, isotretinoin could be effective in the release of hBD through all of these abovementioned mechanisms. Harder et al.[28] evaluated hBD-1, hBD-2, hBD-3, and hBD-4 gene expression stimulation and showed that all-trans retinoic acid inhibited pro-inflammatory cytokine-mediated release of hBD-2, hBD-3, and hBD-4 gene expression which was not observed with hBD-1 expression. Under the light of these data and our finding, a retinoic acid derivative, isotretinoin might be considered to lead to similar effects on hBD-1 and hBD-2, and that isotretinoin showed one of its anti-inflammatory effects through reducing only hBD-2 release.

In our study, a significant difference between isotretinoin and doxycycline groups with regard to posttreatment hBD-1 and hBD-2 level was not found. We concluded that isotretinoin and doxycycline had same effect on hBD-1 and hBD-2 release although they do not work through the same mechanisms. Detecting a significant reduction in hBD-1 level with doxycycline treatment

| Table 2: Comparison of the amount of human beta‑defensin‑1 and human beta‑defensin‑2 staining in pustular lesions and in uninvolved skin of acne vulgaris patients with those of the control group |
|---------------------------------|-----------------|----------------|
|                                | Acne patients (n=44) | Controls (n=20) |
|                                | Uninvolved skin    | P               | P                |
|                                | Pustular lesion    | P               | P                |
| Pre‑T hBD‑1, median (minimum–maximum) | 2 (0-3)            | 2 (0-3)         | 0.104<sup>1</sup> | 1 (0-2) | 0.0001<sup>1</sup> |
| Pre‑T hBD‑2, median (minimum–maximum) | 0 (0-1)            | 1 (0-1)         | 0.0001<sup>2</sup> | 0 (0-1) | 0.032<sup>2</sup> |

<sup>1</sup>Kruskal–Wallis test, <sup>2</sup>Mann–Whitney U-test. 0: Negative, +1: Mild, +2: Moderate, +3: Severe, hBD‑1: Human beta‑defensin‑1, hBD‑2: Human beta‑defensin‑2, Pre‑T: Pretreatment, Post‑T: Posttreatment

| Table 3: Effects of isotretinoin and doxycycline treatment on human beta‑defensin‑1 and human beta‑defensin‑2 staining in pustular lesions and in uninvolved skin of acne vulgaris patients |
|---------------------------------|-----------------|----------------|
|                                | hBD‑1           | hBD‑2           |
|                                | Pre‑T | Post‑T | P     | Pre‑T | Post‑T | P     |
| Isotretinoin, median (minimum–maximum) | 2 (0-3) | 1 (0-3) | 0.122 | 1 (0-1) | 0 (0-2) | 0.003 |
| Doxycycline, median (minimum–maximum) | 2 (1-3) | 1 (0-3) | 0.029 | 0 (0-1) | 0 (0-2) | 0.334 |

Pre‑T: Pretreatment (n=22), Post‑T: Posttreatment (n=21), hBD‑1: Human beta‑defensin‑1, hBD‑2: Human beta‑defensin‑2
which did not have a direct effect on sebum release but had a more evident effect on pro-inflammatory cytokines suggested that perilesional infiltrate might play a more dominant role on hBD-1 release. However, no difference was observed in hBD-2 levels with doxycycline treatment which was known to inhibit neutrophil chemotaxis. On the other hand, we observed a significant reduction in hBD-2 levels with isoretinoin treatment which prevented basal sebocyte proliferation and inhibited follicular keratinization. All these might suggest that keratinocyte and sebocytes were more effective than perilesional infiltrate for their contribution in regulating hBD-2 release.

**Conclusion**

According to our results, hBD-1 and hBD-2 levels were elevated in skin with AW lesion and that hBD-1 and hBD-2 had role in acne pathogenesis. Isoretinoin and doxycycline, commonly used systemic agents, are well known to have anti-inflammatory effects. Our findings showing a significant reduction in hBD-1 staining with doxycycline treatment and in hBD-2 with isoretinoin suggested that some part of their anti-inflammatory effect worked through these mechanisms. It was not possible to know for how long the effect of the drugs on the defensin would last. Therefore, in the future, to clarify the effect of isoretinoin and doxycycline treatments on cutaneous defensin release, defensin levels should be measured at different doses of the drugs and should be repeated in follow-up.

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**Conflicts of interest**

There are no conflicts of interest.

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