Special Focus Report

Cutaneous induction of corticotropin releasing hormone by *Propionibacterium acnes* extracts

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**Abbreviations:** *P. acnes*, *Propionibacterium acnes*; *E. coli*, *Escherichia coli*; CRH, corticotropin releasing hormone; FM, membrane fraction; SA, supernatant A; Zn, zinc; LPS, lipopolysaccharide; Ctrl, control; HPA, hypothalamic-pituitary-adrenal; ACTH, adrenocorticotropic hormone; POMC, proopiomelanocortin; CRH-R1, corticotropin receptor type 1; PSU, pilosebaceous unit; PBS, phosphate-buffered saline; TBS, tris-buffered saline; DMEM, dulbecco’s modified eagle medium; TNFα, tumor necrosis factor alpha; IL-1α, interleukin-1 alpha; IL-8, interleukin-8; TLR-2, toll-like receptor-2

**Key words:** acne, *Propionibacterium acnes*, stress, corticotropin releasing hormone, zinc

The skin commensal bacillus *Propionibacterium acnes* is known to play a major role in the development of acne vulgaris and it is established that this bacteria is involved both in the induction and maintenance of the inflammatory phase of acne. The corticotropin releasing hormone (CRH), a neuropeptide originally isolated from the hypothalamus, is also produced by the skin. CRH has been reported to play a role in the inflammation, the production of sebum and finally the differentiation of keratinocytes. At the therapeutic level, zinc is known to act specifically on inflammatory lesions with still partially known mechanisms and thus could play an important role in the development of inflammatory acne lesions. Our objective was to study the modulation of CRH expression by keratinocytes induced by *P. acnes* extracts. CRH expression was examined using immunohistochemistry technique on deep-frozen sections of normal human skin explants incubated with two different extracts of *P. acnes* and with or without zinc salts. We observed that the membrane fraction (FM) of *P. acnes* increased the CRH expression in the epidermis. This result indicates that *P. acnes*, by stimulating the production of CRH, can both modulate the differentiation of keratinocytes and increase the local inflammation, arguing that this bacterium plays a role not only in the development of inflammatory acne lesions but also in the formation of the microcomedo in the early stages of acne.

**Introduction**

Acne vulgaris, the most common pathology of the skin, is a chronic inflammatory disease of the pilosebaceous unit (PSU). Several pathogenic factors such as ductal hypercornification, increased sebum production, abnormality of the microbial flora within the PSU and secretion of mediators of inflammation contribute to the aetiology of this multifactorial disease.1 Although *P. acnes* has been associated with acne, the precise mechanisms governing the development and progression of acne remain unclear.

In response to systemic stress, the hypothalamic-pituitary-adrenal (HPA) axis is activated.2 The process begins with the hypothalamic release of corticotropin releasing hormone (CRH) which stimulates the production of adrenocorticotropic hormone (ACTH) and other proopiomelanocortin (POMC) peptides via the activation of CRH receptor type 1 (CRH-R1). It was reported that ACTH is also produced outside the pituitary tissue, including the skin.3-6 Interestingly it has been proposed that skin has an equivalent of the central HPA axis.5,7-10 There is increasing evidence that the cutaneous nervous system modulates physiologic and pathophysiologic effects in the skin.11 Although similar to its systemic equivalent, the cutaneous HPA axis is responsive to local stressors (solar, thermal, chemical, biological, etc.) and resultanty activates the neuronal, endocrine and immune systems in the skin.

As acne is obviously exacerbated under acute or chronic psychological stress11-13 the role of CRH in this pathology appears as an important point in the development of lesions. In humans, CRH is synthesized among others by keratinocytes,14 immune cells15 and human mast cells16,17 under the influence of a stress. Interestingly, CRH is also reported to play a role in the regulation of keratinocytes proliferation and differentiation representing an important step in the early stages of the development of acne lesions. Moreover CRH is known to act on inflammation by inducing the degranulation of mast cells, the release of inflammatory cytokines and the modulation of immune cells.18-20 And finally CRH acts also on sebum production by promoting lipogenesis in human sebocytes and thus by increasing the seborrhoea.21,22 Consequently, CRH could be strongly implicated in the development of acne.

Furthermore, several studies have underlined an important role of *P. acnes* in the pathophysiology of inflammatory lesions.23-29

In this context, we hypothesized that *P. acnes* is able to increase CRH expression by keratinocytes, representing a local stress in acne. We thus evaluated the modulation of CRH...
expression in the epidermis induced by the microbial stressor *P. acnes*.

**Results**

In all cases, we observed that the mean expression of CRH was moderate (1.25 ± 0.50) in control medium and was significantly increased (p < 0.05) in presence of *P. acnes* FM (3.50 ± 1.00). However the slight overexpression detected in the presence of SA (1.75 ± 0.96) was not significant (p > 0.05). This increased expression of CRH was observed after an incubation of three hours with *P. acnes* extracts.

Concerning LPS which is a proinflammatory substance, a slight increase (1.88 ± 0.25) in the expression of CRH was noted while zinc gluconate decreased this expression (0.50 ± 0.50) in comparison with the control medium, for all donors. However the modulations observed were not significant (Figs. 1 and 2).

**Discussion**

In the present work, we demonstrate that *P. acnes* stimulates the production of CRH by keratinocytes. In addition we observe that zinc gluconate used in inflammatory acne decreases the epidermal CRH production.

Concerning *P. acnes* extracts, the stimulating effect is specifically obtained with FM and not with SA. While FM contains *P. acnes* membrane components, in particular peptidoglycan and lipoteichoic acid, Supernatant A (SA) contains only cytosolic proteins. Our result thus confirms that the CRH overexpression is mediated by at least one component of FM, suggesting that *P. acnes* acts on the CRH production through a direct contact with keratinocytes membranes. Notably, the induction of CRH in the presence of FM is more important than this obtained in the presence of LPS which is considered as a reference among proinflammatory substances.

It is widely accepted that inflammatory acne may be mainly mediated by different factors secreted by *P. acnes*, such as lipases which liberate the production of proinflammatory free fatty acids from sebum.\(^{30,31}\) Recently, it has also been described that *P. acnes* triggers anti-microbial peptide and cytokine secretion of keratinocytes in vitro.\(^{23-25}\) *P. acnes*, itself, is able to induce cytokines-like such as TNFα, IL-1α and IL-8.\(^{26,27}\) Moreover the genome sequence of *P. acnes* is known to encode many factors that may have inflammatory potential.\(^{28}\) But, recently it has also been shown that *P. acnes* was able to induce keratinocytes proliferation and filaggrin expression by keratinocytes,\(^{29}\) indicating that this bacterium could play a role not only in the development of inflammation in acne lesions but also in the formation of the comedo.

The role of zinc salts has already been reported in several studies and beneficial effects were described on inflammatory lesions in mild to moderate acne. It has been shown until now that its anti-inflammatory activity has different targets.\(^{32}\) Indeed zinc acts via an inhibition of polynuclear cells chemotaxis.\(^{33}\) The anti-inflammatory effects of zinc could be attributed to its ability to decrease TNFα and IL-6 production\(^{34,35}\) or to inhibit TLR-2 expression by keratinocytes.\(^{36}\) The modulation of integrins expression by zinc is also described.\(^{37,38}\) At the same time, zinc is known to have a direct influence on *P. acnes* development by its antimicrobial activities\(^{39}\) and its ability to reduce the resistance of *P. acnes* strains to erythromycin.\(^{40}\) Finally, zinc is also able to modulate the 5α-reductase, a key enzyme implicated in the transformation of testosterone leading to an exacerbated seborrhoea.\(^{41}\) By these many actions, zinc is able to improve inflammatory acne. In this study, we suggest another mechanism by which zinc salts could be able to improve acne by decreasing the epidermal CRH expression. The fact that the modulation observed with zinc salts was not significant can be mainly due to the low level of basal CRH production in skin control but interestingly the decreased expression of CRH was noted for all the four donors studied. Moreover, the model used was very close to in vivo. In this context, zinc salts could represent a potential new way to counteract acne by targeting CRH.

The potential interest of CRH in acne was recently suggested by the discovery of an overexpression of CRH in the PSU.\(^{21}\) Indeed, CRH has been reported to play several roles potentially strongly implicated in the development of acne lesions. First, as CRH was reported to promote lipogenesis in human sebocytes and thus to increase the seborrhoea, it could represent a key factor in the development of acne.\(^{21,22}\) Moreover, CRH is also known to act as a growth factor in the skin by activating the CRH-R1. CRH is thus described as an activator of keratinocytes differentiation.\(^{42}\) It is also an inhibitor of the early and late apoptosis of many skin cell types such as keratinocytes, dermal fibroblasts and melanocytes.\(^{43}\) Finally CRH has also a pro inflammatory activity.\(^{18}\) Interestingly, it was suggested that CRH could act as a local endocrine mediator that enhances inflammatory responses to bacterial antigens.\(^{44}\)

In this study, we demonstrate for the first time that *P. acnes* acts as a strong local stressor at the origin of CRH overexpression by keratinocytes, in the epidermis. Thus, as CRH modifies the differentiation of keratinocytes, we confirm that this bacterium has a spectrum of action, not limited to inflammation but also in the early stages of the formation of acne lesions.\(^{29}\) This confirms the fundamental role played by this bacterium which can be detected as a local
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pro inflammatory substance, it was used as positive control of the inflammation. LPS was diluted in DMEM (Dulbecco’s Modified Eagle Medium) (Sigma-Aldrich) medium with a final concentration of 1 μg/mL and incubated with cutaneous explants.

Trace element. As zinc salts are an anti inflammatory treatment of acne lesions it was used as negative control. Zinc gluconate (Labcatal, Montrouge, France), was diluted in DMEM with a final zinc concentration of 1 μg/mL and incubated with cutaneous explants.

Skin explants technique. Punches (4 mm in diameter) from abdominal skin of 6 healthy donors, considered as an healthy skin model, were incubated at 37°C in a moist atmosphere in the presence of 5% CO₂ for 3, 6 or 24 hours in DMEM. The medium contained P. acnes extracts at the following concentrations: FM ½ or SA 1/5 or Zn 1 μg/mL. Medium alone was used as a control. After incubation of 3, 6 or 24 h, explants were removed from the culture medium and frozen at -80°C.

Immunoperoxidase. Sections (5 μm thick) were then cut with a cryostat, fixed in acetone at 4°C for 10 min and frozen at

Figure 2. Detection of the expression of corticotrophin-releasing hormone in the skin after an incubation of three hours in the presence of Medium (Ctrl) (A), SA (P. acnes supernatant A) (B), FM (P. acnes membrane fraction) (C), LPS (E. coli Lipopolysaccharide) (D), Zn (Zinc Gluconate) (E). (Magnification x40).

Materials and Methods

Bacterial extracts. Two extracts of P. acnes IP53113T (Pierre Fabre, Toulouse, France) were made available to us. The strain was first described in 1968. The membrane fraction (FM) contained peptidoglycan and lipoteichoic acid. Supernatant A (SA) contained cytosolic proteins. The membrane fractions of the bacteria were resuspended in DMEM (Dulbecco’s Modified Eagle Medium) (Sigma-Aldrich, Saint-Quentin Fallavier, France).

LPS extracted from E. coli 0111:B4 (Sigma, St. Louis, USA), was reconstituted in PBS (Phosphate-Buffered Saline). As LPS is a stressor by the skin and could potentially lead to the development of acne lesions.

In conclusion, CRH represents a new target for P. acnes in the formation of both retential and inflammatory lesions. Zinc gluconate seems to be able to decrease the production of CRH by keratinocytes, thus explaining its role in the improvement of acne. But this point has to be confirmed by further studies.

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Immunoperoxidase. Sections (5 μm thick) were then cut with a cryostat, fixed in acetone at 4°C for 10 min and frozen at
-20°C. The non-specific sites were saturated for 30 min with TBS, 0.1% and very strong labelling (5).

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