Tretinoin: A Review of Its Anti-inflammatory Properties in the Treatment of Acne

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ABSTRACT

Tretinoin has been primarily used for the early stages of acne because of its proven comedolytic end benefits. This article reviews and updates the collective body of evidence of tretinoin in the treatment of acne, which suggests that this drug also possesses a broad range of acne-related immunomodulating properties that are capable of disrupting and hindering the various stages of the inflammatory cascade and the production of proinflammatory factors associated with it.
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Historically, tretinoin (all-trans retinoic acid) has been used as a comedolytic agent to treat mild-to-moderate acne. Used as a stand-alone treatment or in combination with antibacterials, its ability to stimulate the growth of new cells, unclog pores, and promote the normal flow of sebum is well-proven. However, over the years there has been a growing awareness within the professional community that tretinoin also possesses a broad range of modulating properties, which could play a beneficial role in the disruption of the immunoinflammatory cascade of acne vulgaris and the proinflammatory factors associated with it. This review article is a compilation of such data and is intended to provide the researcher and practitioner with a greater appreciation of the expansive role that tretinoin plays in the treatment of inflammatory acne.

INFLAMMATORY ACNE

Acne vulgaris is a chronic inflammatory disease of a pilosebaceous unit that is able to respond to the threat of infection as an immunocompetent organ.[1] It is generally thought that hypercornification, plugging of the follicular opening by sloughed keratinocytes and other debris, comedone formation, excess sebum production, abnormal proliferation of *Propionibacterium acnes*, and leukocyte infiltration combine to set the stage for a localized inflammatory response.[2–6]

The sebaceous follicle is sensitive to any changes that signal the beginning of an inflammatory episode. Although not fully understood, the first sign of subclinical acne is thought to be the microcomedo "lesion" within which abnormal amounts of keratinized cells are produced. Keratinocytes are part of the skin's first line of defense and, in conjunction with sebocytes, produce proinflammatory chemokines and cytokines that alert the host to the presence of danger and attract lymphocytes and T-helper cells to the microbially infected area.[7] This initial event appears to be the onset of an intracellular communication/signaling process that provides various tissues and organs with infectious information that determines what protective immunological steps to take next. If it is a typical acne inflammatory event, an innate (nonspecific) and acquired (antibody-mediated) immune response will occur.

The ensuing overgrowth of *P. acnes* within the plugged follicle results in the discharge of extracellular foreign materials by this organism. These proinflammatory triggers include lipase, which releases cytotoxic free fatty acids from sebum, as well as protease, hyaluronidase, and neuraminidase—all of which can damage the follicular wall, promote leakage into the surrounding dermis, and trigger inflammation.[8–14] *P. acnes* is also capable of producing other undesirable proinflammatory agents, such as highly immunogenic heat shock proteins, porphyrin that

reacts with molecular oxygen to produce toxic oxygen species and free radicals, and highly comedogenic squalene peroxides.[5,15–17]

During the early stages of preinflammatory and inflammatory acne, *P. acnes* releases potent low molecular weight chemotactic factors and chemotactic lipase that find their way into the dermis and surround the affected follicle.[18–23] The latter are capable of attracting various white blood cells to the site of infection, such as neutrophils, lymphocytes, and monocytes.[3,13,19,24–27]

P. acnes also stimulates the production of small polypeptide, proinflammatory cytokines during the onset of infection. They include interleukins (IL) IL-1α, IL-1β, IL-6, IL-8, and IL-12, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor alpha (TNF-α), and interferon-gamma (IFN-γ). These biologically active messenger molecules originate from white blood cells, keratinocytes, and sebocytes in and around the damaged follicle, as the host cross-talks with skin defense and immune cells to fight and ward off the infection.[1,17,28–39]

Along with the above inflammatory challenges, other innate and acquired immune responses are called into play by the host, which add to the cascade of proinflammatory events within the distended, leaky follicle. Immunoactive T lymphocytes (T-cells), CD^{+}4 cells, and CD14^{+} cells infiltrate the inflamed lesion.[1,36,40–43] Activated monocytes transform into macrophages and begin to surround and engulf foreign materials associated with the infectious threat. Intracellulary, phagocytosing neutrophils/macrophages release degradative lysosomal enzymes and toxic chemicals, such as reactive oxygen species, all of which help to break down and remove *P. acnes* and its injurious extracellular products.[44–47] When produced chronically and excessively, these same materials may also rupture the follicle, find their way into the surrounding dermis, and be inflammatory to the host.[5,46,48–51]

Other proinflammatory factors that are thought to be implicated with pathogenic acne are leukotriene B$_4$ (LTB4) and compliment-activated C5-derived neutrophil chemotactic factors, all of which can recruit and activate neutrophils, monocytes, and eosinophils resulting in more inflammation.[40,52–55] A host of other inflammatory agents and events likely add to the proinflammatory fray: toll-like receptors (TLRs), transcription factor activator protein 1 (AP-1), vascular cell adhesion, macrophage degranulation, growth factors, expression of transglutaminase and lipoxygenase, cytokine modulation of metalloproteinase activity in the dermis, prostaglandin synthesis, and the possible release of IL-5.[30,33,36,43,56–62]

At some point, the inflamed lesion may burst and empty its bacterial contents, cytokines, and chemotactic factors into the dermis and epidermis, which is considered to be the most distressing event in the course of inflammatory acne. This continues to exacerbate the cascade of inflammation within and outside the immediate environment of the ruptured follicle. The net clinical result that typically occurs is moderate-to-severe acne characterized by papules, pustules, and, in more inflamed cases, nodules.

TRETINOIN'S COMEDOLYTIC ACTIVITIES AND POTENTIAL ANTI-INFLAMMATORY END BENEFITS

The comedolytic-related activity of tretinoin has been known for many years. Tretinoin a) normalizes the exfoliation of the follicular epithelium within the pilosebaceous unit, b) inhibits transglutaminase activity that results in cellular adhesion, c) prevents follicular plugging, d) leads to drainage and expulsion of excess sebum and *P. acnes*, and e) helps create a more aerobic environment that is less conducive to *P. acnes*.[2,56,63–67] Although they are often thought of as "indirect" effects, the aforementioned therapeutic end benefits can be considered anti-inflammatory, since all of them help to diminish the potential severity and outcome of an inflamed lesion.

PHAGOCYTOSIS AND THE IMMUNOMODULATING PROPERTIES OF TRETINOIN

Phagocytosis plays a key role in the defense against bacterial infections including acne. Although inflammatory by nature, it is a necessary part of the host's mechanism of protection. When taken as a whole, some authors contend that homeostatic phagocytosis might be considered an anti-inflammatory response since removal of harmful bacteria, their extracellular byproducts, and other proinflammatory debris is beneficial to the host.[56,68,69] Others suggest that during bouts of excessive inflammation, phagocytes can break down and release inflammatory lysozymes and various reactive oxygen species, which can exacerbate inflammation.[44–47] A recent article points out that macrophages can initially add to the inflammatory process, but, when activated to another form, may actually aid in the healing process. This would explain how untreated inflamed lesions resolve favorably and return to normalcy on their own.[70] Overall, the net effect of macrophages in the role of infection may very well be considered a positive one and is treated as such in this article.

Five different inflammatory events associated with acne and phagocytosis are phagocytic function, respiratory burst, inducible nitrous oxide synthase (iNOS) activity, degranulation, and proteolytic enzyme activity. Available data suggest that tretinoin beneficially modulates all of them.

Phagocytic function. Retinoic acid has been shown to modulate *in-vitro* macrophage function. When immuno-globulin G-sensitized bovine erythrocytes were exposed to a mouse macrophage cell line, tretinoin increased phagocytosis more than twofold at low concentrations (5 x 10^{-11}M) and higher when compared to controls. The authors state that they have also observed similar results *in vivo*.[71]

Respiratory burst. Several studies have been conducted which showed that tretinoin is able to suppress respiratory burst and its release of cytotoxic oxygen species, such as superoxide anion and hydrogen peroxide, which could result in less tissue irritation.[59,60,72,73] The first study employed human polymorphonucleocytes and compounds known to activate a respiratory burst response. At nontoxic concentrations, tretinoin produced a dose-dependent (1 to 100µM) inhibition of superoxide anion that reached suppression levels up to 90 percent. The investigators

suggest that tretinoin's dampening effect could explain, in part, its anti-inflammatory properties.[59] A second study showed that tretinoin produced a medium-to-potent inhibition of oxidative burst in rabbit-derived poly-morphonucleocytes.[60] And two *in-vitro* studies showed that tretinoin significantly inhibited the release of oxygen free radicals and the generation of superoxide anions.[72,73]

Nitrous oxide synthase. Proinflammatory cytokines are able to upregulate the cellular expression of nitric acid synthase (iNOS enzyme).[73] Using various forms of this enzyme, keratinocytes and macrophages can produce nitric oxide, which is an important mediator for inflammatory reactions and may even be responsible for triggering the inflammatory cascade.[15]

Tretinoin is known to suppress the release of cytotoxic nitric oxide. Using activated human keratinocytes, investigators showed that tretinoin was able to downregulate the production of nitrites (reflecting nitric oxide pathway activation) up to 70 percent in a dose-dependent manner. In the same test model, the investigators also showed that this same retinoid inhibited the induction of iNOS by 60 percent and suppressed the expression of iNOS messenger RNA (mRNA) by 50 to 70 percent.[74] In a similar study several years later, investigators determined the effect of tretinoin on nitric acid release, induction of iNOS, and expression of iNOS mRNA using human keratinocytes and immuno-globulin E as the induction agent. The results showed a time- and dose-dependent inhibition effect, with the highest levels of suppression being 60 percent for iNOS activity and, after 24 hours of treatment, virtually eliminated the expression of iNOS mRNA.[75]

Degranulation. Degranulation occurs when intracellular granules from phagocytic cells, such as immunocytes and neutrophils, release degradative lysozymes that can digest infectious organisms including *P. acnes*, which can be irritants if released. Two *in-vitro* studies showed that tretinoin inhibits degranulation in phagocytic cells resulting in a diminished release of cytotoxic, tissue-irritating materials.[50,59]

Proteolytic enzymes. During phagocytosis, macro-phages engulf and degrade harmful micro-organisms using proteolytic enzymes. The latter can find their way into the surrounding microenvironment of the acne lesion and cause inflammation. The inhibitory effect of tretinoin on proteolytic enzyme release in human neutrophils showed that increasing concentrations of this drug (5 to 20µg) resulted in correspondingly smaller levels of the degradative enzymes when compared to untreated controls.[73]

TRETINOIN'S CYTOKINE IMMUNOMODULATING EFFECTS

Cytokines are transient immunomodulating substances secreted by specific cells of the immune system. Capable of carrying messages between local cells via signaling pathways, they regulate the duration and amplitude of the immune response. Normally found in unstressed sebaceous glands, their levels increase dramatically when an infectious *P. acnes* challenge occurs and they become key players in the ensuing inflammatory cascade.[76–78]

IL-5 is a cytokine that appears to play a beneficial role in the immunomodulatory process. It has been suggested that upregulation of IL-5 can activate specific T-helper (Th) lymphocytes, such as Th2 cells. These cells, based on their cytokine profiles, may actually aid in the healing of acne lesions. Tretinoin has been shown to activate IL-5 in human peripheral blood mononuclear cells.[62]

IL-6 is a proinflammatory mediator that may play an important role during the early stages of acne. Macrophages, T-cells, and keratinocytes can produce IL-6 during an infectious challenge.[35,79] Using human epidermoid carcinoma A431 cells, tretinoin exhibited a "very potent" inhibition of the phorbol 12-myristate 13-acetate-stimulated release of proinflammatory IL-6 when compared to control cells.[62] A second study confirmed these observations when CD14+ monocytes, the predominant form of TLR2+ cells found in the study of acne lesions, were exposed to heat-killed *P. acnes*. Tretinoin demonstrated a 74-percent inhibition of the release of IL-6 in the monocytes.[43]

IL-12 is produced by macrophages in the presence of antigenic challenge. During an inflammatory episode, it stimulates the growth and function of various immunocytes (especially T cells), the production of IFN-γ and TNF-α, and plays an important induction role in the Th1 immune response. It has been shown that mouse macrophages can produce IL-12 when stimulated with a known lipopolysaccharide (LPS) antigen or the heat-killed pathogenic organism, *Listeria monocytogenes*. When these same macrophages were pretreated with varying amounts of tretinoin, the induction of proinflammatory IL-12 was significantly reduced in a dose-dependent manner, decreasing from approximately 840pg/mL to about 420pg/mL when compared to untreated controls. Administration of tretinoin also caused mouse macrophages to increase production of IL-4 by antigen-primed CD4+ T cells and helped these cells differentiate towards a Th2 phenotype, known to promote healing.[80]

IL-12 is composed of p35 and p40 protein subunits. IL-12-p40 proinflammatory mediators can be released in varying amounts independent of IL-12 and can induce the Th1 helper cell inflammatory response. A recent *in-vitro* study measured the inhibitory effects of tretinoin on the secretion of IL-12-p40 in human monocytes using heat-killed *P. acnes* to activate these white blood cells. Results showed that tretinoin was able to downregulate the induction of IL-12-p40 by 91 percent.[43]

TNF-α, produced in part by human keratinocytes, is a multifunctional cytokine that can induce a broad range of secondary proinflammatory effects in response to microbial infections. It can activate cell adhesion molecules; upregulates prostaglandins, collagenase, various inflammatory cells, and human acute monocytic leukemia cell line (ThP-1) cells; and can induce the release of other cytokines, such as IL-1, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor.[28,31,39]

Several studies have shown that tretinoin is a potent inhibitor of TNF-α production.[43,74,75] The first study evaluated

the effects of tretinoin on the release of TNF-α in CD14+ human monocytes, wherein heat-killed *P. acnes* served as the antigenic stimulant. Results showed that tretinoin was able to downregulate the induction of TNF-α by 70 percent.[43] In a second study, investigators evaluated the effects of tretinoin on the production of TNF-α secretion by human keratinocytes activated with lipopolysaccharides and IFN-γ. In a dose-dependent fashion, tretinoin inhibited the release of TNF-α up to 60 percent.[74] Another similar investigation measured the inhibitory effect of tretinoin on TNF-α release by human keratinocytes. Immunoglobulin E was used as the induction agent to stimulate the release of TNF-α. Results showed a time- and dose-dependent inhibition effect, with the greatest level of TNF-α inhibition being approximately 70 percent.[75]

IFN-γ, another potent cytokine, primes macrophages making them cytotoxic, increases antigen presentation of macrophages, induces the production of E-selectin on endothelial cells, and the production of intercellular adhesion molecule-1 and human leukocyte antigen complex (HLA-DR) on keratinocytes. All of these actions play an important role in the proinflammatory attraction of leukocytes to the site of infection.[81–83] The *in-vitro* inhibitory effect of tretinoin on IFN-γ expression was evaluated in normal human peripheral blood mononuclear leukocytes activated in the presence of *Staphylococcus* enterotoxin B. Tretinoin "very potently" inhibited the release of the proinflammatory cytokine, IFN-γ.[62] A second study evaluated the effect of tretinoin on the induction of IFN-γ by mouse macrophages, which were stimulated with LPS antigen or a heat-killed pathogenic organism, *Listeria monocytogenes*. Macrophages pretreated with varying concentrations of tretinoin exhibited approximately one-half the induction of proinflammatory IFN-γ compared to control values.[80]

TRETINOIN'S EFFECT ON OTHER IMPORTANT PROINFLAMMATORY EVENTS

Other important proinflammatory events take place during the inflammatory cascade, all of which may be beneficially modulated by tretinoin. LTB4 is a proinflammatory agent that is able to recruit neutrophils and other immunocytes to the site of infection and plays a role in stimulating the production of various proinflammatory cytokines and other bioactive mediators. Retinoids have been shown to be potent inhibitors of rat leukocyte LTB4 production *in vitro*. The following three methods were used to measure the effect of tretinoin on LTB4 release in rat leukocytes: 1) polymorphonucleocyte aggregation, 2) polymorphonucleocyte chemokinesis, and 3) physico-chemical measurements as reverse phase-high pressure chromatography. All three methods showed that tretinoin was a potent inhibitor of LTB4 production.[84]

T helper cells, such as CD4+ T-helper lymphocyte cells are among the earliest to infiltrate the lesion during the early stages of acne inflammation. The Th1 subset of these cells are known to secrete proinflammatory cytokines, such as IFN-γ, TNF-α, and others.[62,80] It has also been suggested that the subset of Th2 helper cells might provide healing

influences in acne lesions.[62,85] In a study that evaluated the effect of tretinoin on the expression of IL-5 and TNF-α, it was found that the production of IL-5 was significantly increased while that of TNF-α was inhibited. The investigators suggested that this dual effect could in turn increase the influence of Th2 cells, reduce the response of Th1 cells, and thereby promote healing.[62]

Immunocyte toll-like receptors recognize and identify molecular patterns that occur on invasive bacteria after which they upregulate the induction of IL-1 and helper T cells.[35,56] Several studies have demonstrated that tretinoin can suppress the induction of toll-like receptors.[43,86] One study measured the effects of tretinoin on the expression of toll-like receptor-2 (TLR-2) receptor sites in human monocytes. Results showed that tretinoin was able to downregulate the activation of TRL-2 by approximately 65 percent as measured by the decrease in mRNA that controls this expression. The activation of the coreceptor of TRL-2, known as CD14, was also inhibited by 34 percent. However, tretinoin had no effect on the expression of TRL-1 and TRL-4 receptor sites.[43] A second *in-vitro* study showed tretinoin could suppress the expression of TRL-2 in human monocytes. The investigators initially determined that TRL-2 receptor site was expressed by these immune cells and that the TRL-4 site was not. The authors state, in part, that "all-trans retinoic acid was also active" in suppressing the expression of TRL-2 in human monocytes.[86]

Transcription factor AP-1 is used by modulators of inflammation to control the orchestration and expression of several genes involved with the inflammatory process. Two of these genes encode for vascular endothelial growth factor and matrix metalloproteins. An *in-vitro* study measured, in part, the effects of tretinoin on the expression of transcription factor AP-1. Results showed that the extent of inhibition by tretinoin approached 60 percent.[57]

Cell adhesion protein molecules bind leukocytes to endothelial cells at the site of infection during an initial infectious microbial challenge. It is one of the first defensive immune steps taken by the host. Three different cell adhesion protein molecules are involved in this process, one of which is vascular cell adhesion molecule-1 (VCAM-1). The regulated expression of cell adhesion molecules plays a central role in the initiation of acute and chronic inflammatory disorders. Investigators evaluated, in part, the effect of tretinoin on TNF-α–induced expression of VCAM-1 by cultured dermal microvascular endothelial cells. The authors concluded that "The specific inhibition of cytokine-mediated VCAM-1 gene expression *in vitro* may provide a potential basis by which retinoids exert their biological effects at sites of inflammation *in vivo*."[58]

Prostaglandins can have both proinflammatory and anti-inflammatory properties. Prostaglandin E2 (PGE-2) is released by blood vessel walls in response to infection or inflammation and is typically associated with the inflammatory response. Investigators studied the *in-vitro* production of inflammation-related PGE-2 in cultured cortical astrocytes, which are immune-competent cells found in the central nervous system. LPS was used as an

TABLE 1. Immunomodulating effects reported for tretinoin		
PROINFLAMMATORY FACTORS AND COMPONENTS	**TRETINOIN INHIBITS***	**TRETINOIN ENHANCES/ IMPROVES***
Phagocytosis Related		
Opsonization of bacteria/enhanced phagocytosis	—	—
Respiratory burst (ROS as O_2-, H_2O_2)	X[59,60,72,73]	X[71]
iNOS enzymes	X[74,75]	—
Degranulation/cytotoxic lysozyme release	X[50,59]	—
Proteolytic enzyme activity	X[73]	
Proinflammatory Cytokines		
IL-5	—	X[43,62]
IL-6	X[43,62]	—
IL-12p40/IL-12	X[43,80]	—
TNF-α	X[43,74,75]	—
IFN-γ	X[62,80]	—
Other Proinflammatory Mediators/Events		
Lukotriene (e.g., LTB4)	X[84]	—
T helper cells CD4+ Th2	—	X[62]
Toll-like receptor (TLR) expression	X[43,86]	—
Transcription factor AP-1	X[57]	—
Vascular cell adhesion molecule-1	X[58]	—
Prostaglandin synthesis	X[87]	—
Matrix metalloproteinase	X[88]	—

ROS=reactive oxygen species; O_2-=superoxide; H_2O_2=hydrogen peroxide; IFN-γ=interferon-gamma; TNF-α=tumor necrosis factor-alpha

*Note that tretinoin can enhance rather than inhibit a process associated with inflammation. These enhancements may translate into beneficial immunomodulatory effects. As such, they have been classified as anti-inflammatory.

supportive structure of the extracellular matrix of tissues. When expressed excessively, they can be inflammatory. It has been shown that *P. acnes* induces the production of several MMPs, MMP-9 and MMP-1, in primary human monocytes. *In vivo*, it has been demonstrated that dermal and perifollicular cells can infiltrate acne lesions and express potentially inflammatory MMP-1 and MMP-9 proteases. In this same study, investigators examined, in part, the *in-vitro* role that tretinoin might play in the regulation of MMPs. Monocytes exposed to this retinoid significantly decreased the production of MMP-9. When a mixture of monocytes, tretinoin, and *P. acnes* was evaluated, tretinoin inhibited the expression of MMP-9 and MMP-1, while simultaneously increasing the level of TMP metalloproteinase inhibitor 1 (TMP1). The authors noted that, "These data indicate that *P. acnes*-induced MMPs and TMPs may be involved in acne pathogenesis and that retinoic acid modulates MMP and TMP expression, shifting from a matrix-degradative phenotype to a matrix-preserving phenotype."[88] Table 1 summarizes the immunomodulating observations described for tretinoin.

CONCLUSION

The topical use of tretinoin as an anti-acne agent began almost a half century ago. Since that time it has been successfully used to treat comedonal and inflammatory acne. Over the intervening years, the beneficial effects of tretinoin have grown from an understanding of its potent comedolytic-related properties to an evolving appreciation of its anti-inflammatory actions.

This extensive review of tretinoin's immunomodulating properties suggests that it possesses five important comedolytic-related properties that can be considered anti-inflammatory in nature, as well as 17 other potential activities that may beneficially temper the inflammatory cascade of acne and the production of inflammatory vectors associated with it.

In more recent times, tretinoin has been combined with antibacterials, such as erythromycin, benzoyl peroxide, and clindamycin, to form topical combination treatments for acne. In a recent review of clindamycin's possible anti-inflammatory properties, as many as 14 possible activities

antigen to activate the cortical astrocytes and the latter's array of PGE-2 synthesizing enzymes. Under these conditions, the production of PGE-2 was dramatically increased. However, when repeated in the presence of retinoic acid, the production of proinflammatory PGE-2 was inhibited by about 60 percent.[87]

Matrix metalloproteinases (MMPs) break down the

were found that could have immunomodulating effects, any combination of which may beneficially influence the inflammatory response in acne vulgaris.[89] The topical use of clindamycin and tretinoin as a combination treatment modality that includes antibacterial, comedolytic, and anti-inflammatory properties has proven to be a very effective therapy for treating the various stages of acne.[90–96] It is now becoming increasingly clear that there may be good reasons for these observations.

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