Kallikrein 5-Mediated Inflammation in Rosacea

Clinically Relevant Correlations with Acute and Chronic Manifestations in Rosacea and How Individual Treatments May Provide Therapeutic Benefit

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ABSTRACT

Rosacea is a chronic inflammatory condition of facial skin estimated to affect more than 16 million Americans. Although the pathogenesis of rosacea is not fully understood, recent evidence *in vitro* as well as *in vivo* has supported the role of increased levels of the trypsin-like serine protease, kallikrein 5, in initiating an augmented inflammatory response in rosacea. The increase in the quantity and magnitude of biological activity of kallikrein 5 leads to production of greater quantities of cathelicidin (LL-37), an antimicrobial peptide associated with increases in innate cutaneous inflammation, vasodilation, and vascular proliferation, all of which are characteristic features of rosacea. In this article, the authors review the literature supporting the role of kallikrein 5 in the pathophysiology of rosacea, including how therapeutic interventions modulate the effects of kallikrein 5, thus providing further support for this pathophysiological model that at least partially explains many of the clinical features of cutaneous rosacea.
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Cutaneous rosacea (rosacea) is a chronic inflammatory facial skin disorder noted most commonly in individuals of northern European descent, although people of any ethnicity or skin color may be affected.[1–4] The visible manifestations with central facial predominance are characteristic of rosacea, including erythema, papules, pustules, telangiectasias, and phymatous changes.[1–4] However, persistent (nontransient) erythema involving the central face that intensifies during flares and the presence of telangiectasias, which are also accentuated mostly on the central face, are the core clinical features that support a diagnosis of rosacea.[1–9] Papules and pustules are not consistently present in rosacea, characterizing only those individuals with rosacea who exhibit the papulopustular subtype of the disease.[3–6] In fact, papulopustular lesions never emerge in many individuals affected by rosacesa, and phymatous changes affect only a relatively small number of the rosacea-affected population; however, central facial erythema is present to some extent in essentially all people with rosacea.[1–8]

Why do some people get rosacea and others do not? Although the entire explanation that would fully answer this question remains elusive, current evidence suggests that individuals affected by rosacea exhibit rosacea-prone skin, which inherently displays dysregulation of two main systems present within skin—the neurovascular/neuroimmune system and the immune detection/response system (innate immunity).[3,5–8,10–19] Both of these systems normally serve physiological functions related to how skin responds to exogenous changes or insults (i.e., changes in temperature, exposure to microbial pathogens). However, in rosacea, both the cutaneous neurovascular/neuroimmune system

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and the immune detection/response system are dysregulated, with both demonstrating augmented responses that correlate with clinical manifestations commonly seen in patients with cutaneous rosacea.

Neurovascular/neuroimmune dysregulation, which includes both anatomic and physiochemical differences present in rosacea-prone skin as compared to healthy facial skin, appears to be a major contributor that exacerbates the vasodilation of facial skin vasculature with increased facial blood flow that occurs during a rosacea flare.[3,5–7,17,18,20] This increased vasodilation in rosacea-affected skin, which can be acute or subacute in onset, is commonly referred to as flushing.[1,3,4,6,7,17,20] Neurosensory symptoms (i.e., stinging, burning) are often associated with or exacerbated during a rosacea flare.[1,3–8] Exogenous factors that are commonly recognized by patients as triggers, which seem to induce a flare, include increased ambient heat/warmth and certain spices (i.e., capsaicin), all of which can induce signaling of neurogenic inflammation via specific receptor channels (transient receptor potential vanilloid [TRPV] subfamily) shown to be increased in rosacea-prone skin.[3,6,17,18] The immune detection/response dysregulation of rosacea is evidenced by the upregulation of the pattern recognition receptor, toll-like receptor 2 (TLR2) and the cathelicidin innate immunity pathway.[3,5–17,19,21,22] Ultraviolet light (UV) exposure, another recognized trigger factor associated with flares of rosacea, produces changes that induce ligand-binding of TLR2, which signals innate inflammation.[3,5–16,19,21] Lastly, upregulated production of several matrix metalloproteases (MMPs) has been demonstrated in rosacea, further contributing to cascades of inflammation and degradation of the dermal matrix.[1,3,5–7,19] Accentuated immune detection/response as a major component of the pathophysiology of rosacea has been discussed extensively in the literature and is addressed in more detail as a major subject of this article.[5–7,10–16,19,21,22]

Although the pathophysiology of rosacea is not completely understood, dysregulation of the innate immune detection/response system plays a significant role in the inflammatory and vascular responses seen in this condition.[5–7,10–16,19,21,22] As a known inducer of innate and cellular inflammation, increased vascularity, and angiogenesis, cathelicidin (LL-37), an antimicrobial peptide that physiologically provides near-immediate innate defense against several microbial organisms, has been investigated to determine its potential role in the pathophysiology of rosacea.[10,11,23,24] Results have shown that patients with rosacea express elevated levels of LL-37 in facial skin, with this increased expression attributed to abnormally high levels of the trypsin-like serine protease enzyme, kallikrein 5 (KLK5), which selectively cleaves an inactive precursor protein (hCAP18) to form the biologically active antimicrobial peptide (LL-37).[10,22] Investigations of the mechanism of action of two agents proven to be effective in reducing papulopustular lesions and perilesional erythema in rosacea, topical azelaic acid (AzA) and oral doxycycline, demonstrated direct and indirect inhibition of KLK5, respectively.[25–29] In one study with AzA 15% gel, the reduction in KLK5 activity correlated with clinical improvement of rosacea.[29] In this review, the authors further describe the role of KLK5 in the pathophysiology of rosacea, including the inflammatory cascades that result from increased KLK5 expression, as well as a more detailed discussion of different therapies shown to inhibit the progression of this cascade.

THE PATHOPHYSIOLOGICAL ROLE OF KALLIKREIN-5 IN ROSACEA

KLK5, a member of the kallikrein family of proteases, is the primary serine protease responsible for the cleavage and activation of cathelicidin (LL-37).[10,15,22] In lesional rosacea skin, KLK5 levels are increased, leading to increased levels of both LL-37 and its proteolytic fragments.[10–12,15] In addition to their increased abundance, these peptides also differ from those found in normal individuals. Unlike normal LL-37 peptide fragments, these abnormal peptides control functions, such as leukocyte chemotaxis, angiogenesis, and expression of extracellular matrix components.[23,24,30] Their role in rosacea was confirmed by injecting these peptides into mouse skin, which led to an inflammatory response similar to that seen in patients with rosacea.[10] Together, these findings suggest that the pathophysiology of rosacea may be initiated by an abnormal innate immune response that induces abnormally high levels of KLK5 that cleave larger precursor peptides into LL-37 at very high concentrations, along with multiple variant peptides not present in normal skin. The increased levels of LL-37 and other peptides induce the clinical manifestations seen in rosacea, including inflammation and increased facial vascularity. Therefore, medications blocking the activity or inhibiting the production of KLK5 should prevent the formation of LL-37 and abnormal peptide fragments, thus inhibiting a major cascade of inflammation that has been correlated with major clinical findings in rosacea (Figure 1). As LL-37 has also been shown to promote vascular proliferation in some research models, continued suppression of its overexpression may potentially reduce the development and progression of increased superficial facial vascularity over time. However, further research is needed in people affected by rosacea to confirm that therapies that reduce serine protease activity (SPA) can mitigate the continued development and further progression of the fixed centrofacial vascular changes of rosacea, which produce diffuse persistent nontransient facial erythema of rosacea.[5,8,11,12,20,24]

TOPICAL AZELAIC ACID IN ROSACEA

Since its approval by the United States Food and Drug Administration (FDA) in 2003 for the topical treatment of papulopustular rosacea, azelaic acid (AzA) 15% gel has proven to be safe and effective in the treatment of papulopustular rosacea.[25,27,29] Several mechanisms of action (MOAs) have been proposed to explain why AzA is effective in treating papulopustular rosacea, including antimicrobial effects, decreased keratin production, and inhibition of the formation of reactive oxygen species.[31] However, none of these MOAs have been evaluated specifically in patients with rosacea or in any other relevant surrogate research models.

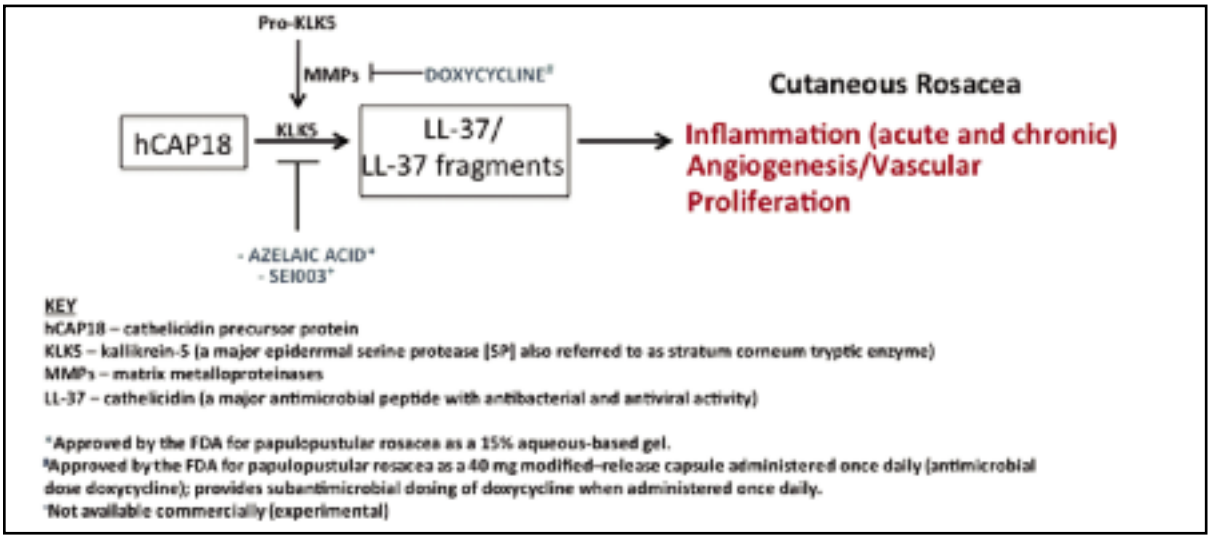

Figure 1. Schematic depicting the inflammatory cascade seen in rosacea. Increased cathelicidin and KLK5 levels lead to the pro-inflammatory and pro-angiogenic states seen in rosacea. Over time, vascular proliferation progresses leading to altered cutaneous vasculature characterized by an increase in the number and caliber of superficial cutaneous blood vessels. These vascular changes, which develop over time, appear to account for telangiectasia formation and diffuse persistent facial erythema of rosacea that worsens during a flare and persists between flares of the rosacea.

More recent studies suggest that AzA may exhibit another MOA that is operative in the treatment of rosacea, specifically by suppressing key steps in the cathelicidin pathway.[29]

An *in vitro* study on cultured keratinocytes showed that AzA directly inhibits KLK5 protein expression.[29] These results were confirmed *in vivo* in a mouse model as well as in human subjects. Mouse skin treated with AzA 15% gel for nine days had decreased KLK5 messenger ribonucleic acid (mRNA) expression as measured by quantitative real-time polymerase chain reaction compared to skin treated with vehicle control (P=0.01920).[29] Application of AzA 15% gel also suppressed expression of Camp and TLR2, although when compared to results noted after vehicle application, the magnitude of difference was less than with KLK5 expression.[29] In another study, adults with papulopustular rosacea of at least mild severity who applied AzA 15% gel twice daily had decreased cathelicidin mRNA expression after four weeks and decreased KLK5 mRNA expression after 12 weeks.[29]

Given the role of KLK5 as the predominant serine protease in the epidermis, a multicenter study of adult patients with papulopustular rosacea (N=55) of at least mild severity investigated SPA in patients treated with AzA 15% gel twice daily.[29] A defined tape-stripping technique was used to obtain stratum corneum specimens from the central and lateral face at baseline and every four weeks through Week 16, with mapping utilized to optimize consistency of sample collection. Compared to control subjects, rosacea patients had increased SPA, with two subsets identified based on SPA at baseline. One subset group demonstrated high SPA and the other low SPA, with high SPA correlating directly with greater severity of both erythema (based on Clinician's Erythema Assessment [CEA]) and overall rating of rosacea severity at baseline (Investigator's Global Assessment [IGA]). It is important to recognize that as the target enrollment of subjects for this study was relatively small, it was not designed to include a large population of patients with severe IGA or CEA baseline values. However, there was a clear trend suggesting that both the severity of rosacea at baseline and the response to treatment with AzA 15% gel correlated with higher SPA values. Ultimately, additional studies will be necessary to confirm the role of SPA measurements in rosacea, to further evaluate subsets of SPA among the rosacea population and their clinical significance, and to determine whether or not SPA can serve as an accurate predictor of therapeutic response. The results of this study did show that facial application of AzA 15% gel twice daily in adults with papulopustular rosacea inhibited SPA activity and reduced both cathelicidin and KLK5 expression.[29]

OTHER SERINE PROTEASE INHIBITORS AND ROSACEA

Other direct serine protease inhibitors have also been shown to inhibit KLK activity and improve the clinical features of rosacea.[32] In this study, ε-aminocaproic acid (ACA), a serine protease inhibitor approved by the FDA for systemic administration in cases of fibrinolytic bleeding, was first tested *in vitro* against the known serine protease inhibitor aprotinin to determine its ability to inhibit KLK5 activity. Compared to vehicle, both ACA and aprotinin significantly inhibited KLK activity. ACA (2M) was then compounded with an equal weight of a base cream and formulated as SEI003 for testing in a randomized, double-blind clinical trial on 11 patients with papulopustular rosacea. Results of these studies showed that patients randomized to the SEI003 group had decreased SPA compared to the group receiving the base cream plus ACA vehicle alone six weeks

after the trial began. In addition, patients in the SEI003 group had a reduction in their papule count and erythema scores at Week 12 compared to those in the control group.[32] These findings support the hypothesis that inhibiting KLK5 decreases the SPA in patients with rosacea, which in turn is associated with clinical improvement.

ORAL DOXYCYCLINE IN ROSACEA

Oral tetracycline agents have been a mainstay in rosacea therapy for decades, with doxycycline being the most extensively studied.[2,8,26,27,33] As it exhibits antibiotic properties, doxycycline was assumed to treat rosacea by eradicating microbes on the skin surface that were thought to contribute to the pathogenesis of rosacea. In more recent years, the antibacterial concept in rosacea has been questioned when subantimicrobial dosing of doxycycline effectively improved signs and symptoms of papulopustular rosacea with reduction of inflammatory lesions, and the biological effects of tetracyclines unrelated to antibiotic activity have been elucidated further and correlated with the pathophysiology of rosacea.[2,3,5,6–8,11,12,19,33,34–39]

Further research *in vitro* using human keratinocyte assays showed that doxycycline indirectly inhibits SPA by inhibiting matrix metalloproteinases that are required for KLK5 activation.[28] Multiple studies have demonstrated the efficacy of anti-inflammatory-dose doxycycline in papulopustular rosacea, a modified-release formulation of doxycycline 40mg administered once daily (doxycycline-MR 40mg capsules once daily) that has been FDA approved for the treatment of papulopustular rosacea since 2006.[26,27,38,40] Decreased LL-37 levels were also shown to be correlated with clinical success in patients treated with doxycycline-MR 40mg capsules once daily, and total protease activity was found to be a predictor of clinical response to this therapy.[41,42] Additional research with doxycycline-MR 40mg capsules once daily used to treat patients with papulopustular rosacea demonstrated clinical efficacy, which similar to results shown with AzA gel 15%, occurred primarily in patients with higher baseline SPA.[41,42] These results further support the roles of LL-37 (cathelicidin) and increased serine protease levels and activity in the pathogenesis of rosacea and again suggest that inhibiting the cathelicidin inflammatory cascade that leads to increased LL-37 levels improves rosacea through either direct or indirect suppression of increased SPA.

DOES COMBINING MODES OF ACTION PROVIDE A RATIONALE FOR COMBINATION THERAPY

It is interesting to note that AzA gel 15% and doxycycline-MR 40mg capsules once daily inhibit the cathelicidin pathway at different points, suggesting the potential for additive or synergistic therapeutic benefit when these therapies are used in combination for papulopustular rosacea. This may be most clinically relevant in papulopustular rosacea that is moderate to severe in magnitude. The available data using a topical agent and oral doxycycline (including sub-antimicrobial dosing) for papulopustular rosacea suggests a more rapid onset and greater magnitude of improvement as compared to monotherapy in studies completed over 12 to 16 weeks.[38,43–46] In addition, prolonged administration of doxycycline-MR 40mg capsules once daily has been shown to maintain anti-inflammatory activity without producing antibiotic effects or inducing antibiotic resistance.[26,38,40]

LIMITATIONS OF CURRENT UNDERSTANDING OF ROSACEA PATHOPHYSIOLOGY AND AVAILABLE THERAPIES

Although there is a body of strong evidence supporting the hypothesis that increased KLK5 expression triggers the cathelicidin inflammatory cascade that is operative in the pathophysiology of rosacea, the fact that none of our current therapies completely clear all of the visible signs and symptoms of rosacea suggests that there is still a lot about the disease and its treatment that are not fully understood. Neurovascular/neuroimmune dysregulation has also been suggested as an important pathophysiological component of rosacea and is supported by scientific evidence of physiochemical and structural changes present in rosacea-prone skin.[3,6,7] In addition, it is possible that there are other immune cells, cytokines, chemokines, peptides/enzymes, receptors and/or neurotransmitters that are contributing to the visible signs and symptoms seen in rosacea that have not yet been recognized or identified. These immunological changes may be part of the inflammatory cascade triggered by increased KLK5 expression, or they may result from activation of separate inflammatory cascades that occur upstream or remote from KLK5 and work in parallel with KLK5 to contribute to the inflammatory response seen in rosacea. This possibility is supported by the fact that there are different subtypes or clinical presentations of rosacea, each of which is associated with different clinical manifestations. It is believed that there is more than one pathway responsible for the clinical changes associated with rosacea and that inhibiting KLK5 blocks one of these pathways.[1–3,5–8] In addition, inter-individual differences in expression of various pathways based on variations in genetic expression may explain the differences in clinical manifestations of rosacea from person to person and over time.[3,6,7]

Similar to the concept that individuals have different clinical manifestations of rosacea, the finding that patients with papulopustular rosacea could be classified into groups with either a high or low baseline SPA suggests that even rosacea-affected people with the same subtype or clinical pattern of rosacea can differ in terms of their physiological responses to the causative immunological dysregulation. The fact that high baseline SPA levels were correlated with more severe disease manifestations supports the role of KLK5 in promoting the inflammatory changes seen in rosacea and also its contributory role in alteration of facial skin vasculature. However, the fact that patients with low baseline SPA did not have a significant change in their SPA after therapy (i.e., AzA) again suggests that these patients may somehow differ from patients with high baseline SPA.

Therapies, such as topical AzA and oral doxycycline, have proven to be effective in papulopustular rosacea with multiple studies demonstrating marked reduction in

inflammatory lesions and associated inflammatory erythema (lesional, perilesional).[2,5–7,25–27,31,33,34,38,40,43–46]

However, these therapies are not curative or always capable of completely clearing the clinical manifestations of rosacea, thus suggesting the presence of other pathophysiological processes, the need for greater magnitude of pharmacological effects from administered therapeutic agents *in vivo*, or both. AzA, SEI003, and doxycycline all typically require weeks of use before visibly apparent results are perceptible and take longer to achieve their maximal therapeutic effect. These findings may reflect difficulty in achieving optimal concentrations of these therapies *in vivo*, requiring longer periods of use before the drug can have a physiological effect, and/or may be a result of the chronic nature of the disease process itself requiring longer treatment durations to induce therapeutic effects on the long-term cutaneous changes associated with rosacea. Fixed changes in the superficial cutaneous vasculature that develop over time appear to account for the diffuse, persistent, non-transient, centrofacial erythema of rosacea that remains between active flares of the disorder.[5,7,9,11,19,24,47] As a result, no single therapy to date demonstrates the ability to resolve all of the clinical manifestations that occur in association with rosacea. A combined approach, often utilizing multiple medical modalities and incorporation of physical modalities (i.e., intense pulsed light, pulsed dye laser, others), is often needed to address the full spectrum of specific clinical manifestations that can affect a given individual with rosacea.[2,5,7,48,49]

DOES CATHELICIDIN PLAY A KEY ROLE IN THE DEVELOPMENT AND PROGRESSION OF CUTANEOUS CHANGES OF ROSACEA OVER TIME?

A final thought that is worthy of consideration is the potential role that cathelicidin (LL-37) may play in the development and progression of cutaneous changes of rosacea over time. As patients experience repeated flares of rosacea, multiple exposures to upregulated expression of LL-37 produces continued signaling of the cascades of inflammation that produce both acute inflammation as well as effects that are more chronic in nature. The latter would include angiogenic effects and fixed changes in superficial cutaneous vasculature that lead to increased skin vascularity with enlarged dilated vessels and telangiectasia.[5–7,11,12,19,24,47] Is it possible that regular and consistent use from the early onset of rosacea of agents which inhibit LL-37 formation by directly or indirectly suppressing SPA could interfere with the development and/or progression of rosacea manifestations? Unfortunately, there is a conspicuous absence of data on the natural history of rosacea and the effects obtained with long-term therapy. Hopefully, further research will provide answers to these and other clinically relevant questions about rosacea and its optimal management.

CONCLUSION

KLK5 is a key mediator of the inflammatory and vascular responses seen in rosacea. Direct and indirect inhibitors of KLK5, including AzA, doxycycline, and SE1003 have all been shown to suppress both the underlying pathophysiology as well as the clinical manifestations of rosacea. As discussed above, abnormally high KLK5 expression is not believed to account for all of the clinical manifestations noted in rosacea-affected patients. The initial trigger causing KLK5 activity to increase may also cause the release of other cytokines or immune cells functioning through different pathways to promote inflammatory and vascular changes seen in rosacea as well as associated symptoms. Neurovascular dysregulation has been described in the literature as an important component of rosacea pathophysiology and is believed to contribute to acute vasodilation and neurosensory symptoms. Further research into KLK5 and rosacea triggers in general may help elucidate these pathways and lead to development of further treatments that may help clinicians optimize the management of rosacea.

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