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INTRODUCTION

ORIGINAL ARTICLE

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Harnessing the Power of Nitric Oxide for Therapeutic Application in Dermatology

Leon H. Kircik MD

Dermatology is a field long associated with innovation. From the early days of the specialty, all the way from using medicated soaps with tar and sulphur to compounding various concoctions, to the ongoing expansion of the biologics today, dermatology has been focused on adopting unique solutions to common problems.

The pace of innovation continues with the development of nitric oxide (NO) as a topical therapeutic agent. This novel investigational molecule for the treatment of acne vulgaris addresses two significant issues. It offers a new approach to managing one of the most common skin diseases we encounter every day in our offices. And it does so while eliminating a growing clinical concern: the rise of bacterial resistance.

Nitric oxide has long been known to provide immunomodulatory and antimicrobial effects when endogenously expressed. However, researchers continue to elucidate a wide range of physiologic and immunologic functions. Only recently have formulators achieved success in harnessing the power of nitric oxide for therapeutic application. A stable, topical formulation that will release an appropriate concentration of NO to the target site had been elusive.

Topical formulations now under investigation show promise for the treatment of acne, and researchers and clinicians have an eye toward other infectious and inflammatory cutaneous conditions, such as wound healing, warts, and onychomycosis. In systemic forms, NO is also under investigation for non-dermatologic indications.

The emergence of NO as a possible topical treatment for acne is significant. Nitric oxide's anti-inflammatory properties are well documented. NO's anti-inflammatory actions include inhibition of IL-1β and Th17 activation. Increasingly, dermatologists are recognizing that acne is essentially an inflammatory skin disease—even if we continue to use imprecise terms like "inflammatory" and "non-inflammatory" to characterize lesions. Similarly, we are recognizing that inflammation underlies virtually all the common presentations we see in the dermatology clinic.

On the other hand, dermatologists continue to grapple with the challenges of antibiotic resistance and their role in reducing it. Even as global guidelines recommend a reduction in use of antibiotics to treat acne, they are still continued to be considered essential to treatment. The data to date show that nitric oxide's antimicrobial effects lead to a reduction in *P. acnes*. Yet, at high concentrations, NO has not been associated with the development of bacterial resistance.

As discussed ahead, topical NO represents an important potential therapeutic option for the treatment of acne and possibly other dermatoses. Now that formulators have overcome the challenges of topical delivery, clinicians can be optimistic that topically applied NO may become available for the safe and effective treatment of acne, warts, onychomycosis, and other inflammatory dermatologic diseases in the near future. Importantly, this novel treatment does not appear to contribute to the global problem of bacterial resistance while providing a novel approach to management of decades old common cutaneous disorders. Once again, dermatology is identifying an elegant solution to our unmet clinical needs.

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Spotlight on the Use of Nitric Oxide in Dermatology: What Is It? What Does It Do? Can It Become an Important Addition to the Therapeutic Armamentarium for Skin Disease?

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ABSTRACT

Nitric oxide (NO) is a diatomic gas that is synthesized within and released by multiple host cell types functioning to provide a variety of physiologic and homeostatic effects. Nitric oxide exhibits a variety of effects that relate significantly with outcomes that can provide therapeutic benefit if properly formulated and released. These include anti-inflammatory, immunomodulatory, antimicrobial, vasodilatory properties, and effects that are beneficial to wound healing. Lack of antibiotic resistance appears to be one major advantage of topically delivered NO. A specific topical formulation of NO has been developed that has been shown thus far in clinical studies to exhibit favorable efficacy and safety. This article provides a thorough review of the biologic effects of NO, discusses modes of action and potential pharmacologic benefits, and reviews currently available clinical data for acne.

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Over the past several years, there has been a conspicuous absence of new therapeutic drug classes and individual compounds developed in dermatology, especially for the topical treatment of skin disorders. Many of the therapeutic advances with topical therapy have primarily involved the development of new vehicles and stabilized combination formulations. Boron-based compounds represent one of the newer areas of drug development in dermatology, which has led to therapeutic agents that are applied topically.[1] Another specific area of drug development has focused on the evaluation of nitric oxide delivery systems, including a variety of topical methodologies.[2,3] The high level of interest in nitric oxide as a therapeutic agent has progressively emerged over approximately two decades as researchers have come to increasingly understand the many physiologic and homeostatic roles that are carried out by this ubiquitous endogenous compound, including many functions within skin.[4-11] The main objective of this manuscript is to provide the reader with a foundation for understanding of what nitric oxide is, how it functions physiologically, and why it may come to serve as an important therapeutic agent in dermatology. As nitric oxide exhibits a diverse spectrum of biologic effects and physiologic functions within multiple organ systems within the human body, emphasis will be placed on understanding relevant mechanisms of action with a focus on dermatologic applications.

What Is Nitric Oxide?

Nitric oxide (NO) is a diatomic molecule that is endogenously expressed by a wide variety of cell types.[4-6,11] When host cells are stimulated to release NO, its activities serve to provide both *antimicrobial and immunomodulatory properties* that are integral to the host immune system.[2,4-6,10,11] Although much of the more recent literature on NO has emphasized its antimicrobial effects, NO exhibits a variety of diverse physiologic and immunologic functions. The production and release of constitutively expressed NO is regulated in order to homeostatically sustain a physiologic balance within multiple individual organ systems, such as those involved in maintaining vascular tone, neuronal transmission, modulation of wound healing, platelet adhesion, and multiple functions in skin, such as the antimicrobial barrier, vascular response (ie, vasodilation), and tissue repair.[5,6,11] Signals produced during microbial invasion are also capable of inducing "on demand" the production and release of high concentrations of NO that are capable of neutralizing a wide variety of bacteria, viruses, fungi, and parasites via several distinct mechanisms that are cytotoxic to most microbes.[4-6,10,11] Hence, controlled NO production and release are vital components of maintaining a functional physiologic balance within skin and other organ systems, and also provide innate protection against both cutaneous and systemic infections. This latter function is supported by studies that demonstrate that NO

deficiency increases susceptibility to infection, and systemic infection is associated with an increase in circulating nitrate and nitrite compounds.[4,5]

The gaseous physiochemical nature and small molecular radius of NO allows it to readily cross cellular membranes at sites where it is released and spreads within contiguous tissues via a high-to-low concentration diffusion gradient.[5,10] The biologic effects produced by NO are both concentration-dependent and duration-dependent, with higher concentrations providing more sudden antimicrobial activity (eg, neutrophil "burst'), and lower concentrations providing signaling and modulation of specific biologic activities mediated by a variety of cell types (to be discussed later).[4-6,10,11] Essentially every cell type is capable of NO expression, including neutrophils, macrophages, keratinocytes, dendritic cells, melanocytes, fibroblasts, endothelial cells, and adipocytes.[5,8,11] As NO is highly reactive and its half-life is very short, both the concentration of NO that is released and the duration of tissue exposure are important factors that impact on its biologic effects.[5,10,11]

Synthesis of NO occurs via the enzymatic activity of one of three isoforms of NO synthase (NOS).[4,5,10,11] As noted above, NO may be constitutively expressed at low levels (<1 μM) to function as a signaling molecule that provides immunoregulatory and antimicrobial activities; low fluxes of NO enhance multiple cellular immunologic activities (ie, proliferation, differentiation, apoptosis), adhesion factor expression, and extracellular matrix synthesis and deposition.[5,11,13] Two endogenous forms of NOS are constitutively expressed, tightly regulated, and characteristically produce NO that is released in short bursts at low flux levels. They are neuronal NOS (NOS1; nNOS) and endothelial NOS (NOS3; eNOS).[5,11,13] A third isoform of NOS, referred to as inducible NOS (NOS2; iNOS), is stimulated by specific microbial products (ie, polysaccharides, endotoxins, cytokines), which "induce" the rapid production and more sustained release of high quantities of NO (>1 μM).[5,11,13] The multiple intermediate derivatives of NO that are present in high concentrations after the release of a large NO flux innately inhibit infection via a variety of modes of action that are cytotoxic to microbial cells, such as nitration, nitrosation, and oxidation (to be discussed later).[4-6,10-13]

What Are the Basic Challenges Related to the Development of Topical Nitric Oxide-Based Formualtions?

The many biologic properties of NO provide a basis for research of potential drug delivery platforms that must prove to provide the necessary stability and release characteristics that harness NO into an effective and safe topical therapeutic agent. As NO is a diatomic gas that is highly reactive chemically and very short-lived after its release into tissues (t ½ ≤1 second), a major challenge has been the development of formulations that are stable when stored and effectively deliver NO to the target site of disease after topical application.[2,3,5,6,10,11,14] However, it is important to first understand the biologic properties of NO and its derivative byproducts. The ultimate goal is to effectively translate these biologic properties into pharmacologic modes of action when delivered to the skin. As the activity of NO is both concentration-dependent and duration-dependent, different delivery systems may potentially influence the therapeutic properties of a given formulation depending on its individual pharmacologic and pharmacokinetic profiles.[2,3,5,6,10-12]

What Properties Support the Consideration of Topically Applied Nitric Oxide as a Therapeutic Agent in Dermatology?

As many dermatologic diseases have been associated with alterations in NO activity, or may involve pathways of inflammation that can be therapeutically regulated by NO, the evaluation of topical NO formulations as potential therapeutic agents in dermatology is both a rational and important endeavor.[5,11,14,15] Although the term "oxidative stress" is often perceived as having a negative connotation, in fact, normal physiology within host tissues incorporates a variety of reduction/oxidation-based (redox) molecules, which are naturally produced to serve as vital components of inherent immunity and tissue repair.[4,5] NO and its derivative reactive nitrogen molecules (RNMs) are among a group of natural redox intermediates that provide "double duty" along with endogenously produced reactive oxygen molecules (ROMs), such as peroxide and superoxide.[5] Thus, NO participates directly in the eradication of microbial pathogens and also contributes to downstream signaling pathways that serve to further modulate immunologic and tissue restorative responses.[4-6,11,15] It is important for the reader to understand that the array of biologic effects and chemical reactivities associated with NO and RNMs in host tissues is highly detailed and complex.[4-6,10] The following serves to summarize the major biologic activities of NO and its derivative byproducts that appear to correlate with potential therapeutic applications in dermatology.

Antimicrobial Properties

Innate antimicrobial activity against a vast array of microbial pathogens has been well described as a major biologic function of NO.[3-6,8,9,11,15-19] Importantly, RNMs and ROMs produced by autoxidation of NO induce activity against various microbial targets through a variety of cytotoxic modes of action which are summarized in Table 1.[3-6,10,11,13,15] The diversity of these mechanisms decreases the capability of microbial pathogens to develop resistance to the antimicrobial effects of NO, especially in the presence of high concentrations of NO and RNMs.[5,15,16]

Studies completed with a variety of NO-based delivery platforms have demonstrated antimicrobial activity against several bacteria and other microbial organisms.[3-5,11,15-21] These include *Staphylococcus aureus* (including methicillin-resistant strains [MRSA]), Group B *Streptococcus spp, Propionibacterium acnes,*

TABLE 1.

Antimicrobial Modes of Action Associated With Nitric Oxide and Its Derivative Byproducts[3-6,10,11]	
Mode of Action	**Antimicrobial Mechanism of Microbial Cytotoxicity**
Damage to Microbial DNA	RMNs produced by autoxidation of NO alter DNA structure, inhibit DNA repair enzymes, and modify proteins through interactions with several amino acids.
Lipid Peroxidation	Microbial lipid damage mediated by exposure to RNM/ROM derivatives of NO (ie, peroxynitrite, nitrogen dioxide).
Binding of Heme Moieties in Critical Proteins	Low concentrations of NO reversibly bind to guanylate cyclase. High NO/RNM concentrations irreversibly bind to the heme moiety of metalloenzymes causing depletion of iron from bacteria.

Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens, Clostridium difficile, Trichophyton spp, Candida albicans, Leishmania spp, and *Trypanosoma cruzi*.[5,11,15-21] Although some microbial organisms have developed mechanisms that may decrease their sensitivity to NO concentrations that are released physiologically, available evidence supports that the high concentrations of NO produced after exogenous application circumvent antimicrobial resistance to NO.[5,11,15,16,22-25]

Cytoprotective Properties

NO has also been reported to provide certain *cytoprotective properties*. These include the following: (1) production of erythema in response to ultraviolet (UV) exposure with protection against endothelial cell apoptosis; (2) melanogenesis secondary to tyrosinase stimulation in response to UV light exposure, which serves to mitigate UV-induced cellular injury; and (3) a relative inhibition of host (mammalian) cell cytotoxicity and lipid peroxidation caused by oxidative injury.[5,6,11-13]

Immunomodulatory Properties

In addition to the antimicrobial properties described above, NO has been associated with immunomodulatory activities that can serve to maintain homeostasis within host tissues and in some cases can provide anti-inflammatory effects and tissue restorative properties that can mitigate pathophysiologic processes.[4,11,15,17,19] Both NO and RNMs can regulate immune response through stimulation of genes that can encode for several transcription factors and regulatory molecules. Examples of immunodulatory effects of NO include regulation of NF-kB and IL-1β activity, inhibition of inflammasome activity, modulation of T-lymphocyte functions, and effects on matrix metalloproteinase enzymes (MMPs), all of which appear to play a role in acne pathophysiology; and modulation of anti-inflammatory cytokines and possibly MMPs involved in wound healing and tissue repair.[4,26-37] The interactions of the several immunomodulatory processes that involve NO and its derivative molecules are summarized in detail elsewhere.[4] Suffice it is to say that specific biologic properties of NO, if captured in the correct delivery platform, have the potential to provide important therapeutic applications in dermatology for cutaneous infections, inflammatory dermatoses (ie, acne), and tissue repair/wound healing.[2,3,5,11,14,15] Table 2 depicts some of the immunomodulatory effects associated with NO and its derivative molecules, emphasizing those that may have applications in dermatology.

What Nitric Oxide-Releasing Delivery Platforms Have Been Evaluated to Date?

As noted above, the physiochemical properties of NO that confound storage stability, and its variable pharmacologic profile based on the concentration released and the duration of target site exposure, provide major challenges in the development

TABLE 2.

Immunomodulatory Modes of Action Associated With Nitric Oxide and Its Derivative Byproducts[5,11,15,19,26-28,40-42]	
Biologic Properties	**Immunomodulatory Effects**
Decreased Production of IL-1β	Inhibition of both caspase-1 and NLRP3 inflammasome activation, which decreases IL-1β expression and provides downstream inhibition of pro-inflammatory cytokine release.
Decreased Production of IL-17	Inhibition of Th17 helper cell proliferation and function; Induction of T-regulatory (Foxp3 negative) cells that inhibit Th17 cell activity.
Decreased E-selectin Expression on Endothelial Cells	Suppression of lymphocyte diapedesis resulting in decreased trafficking of inflammatory cells to the perifollicular region; decreased expression of vascular adhesion molecules inhibits perifollicular inflammation.
Regulation of Matrix Metalloproteinase Activity and Anti-Inflammatory Cytokines	May affect dermal matrix tissue restoration through modulation of MMPs and cytokines associated with wound healing and tissue repair (ie, IL-4, IL-13, IL-10, TGF-β).

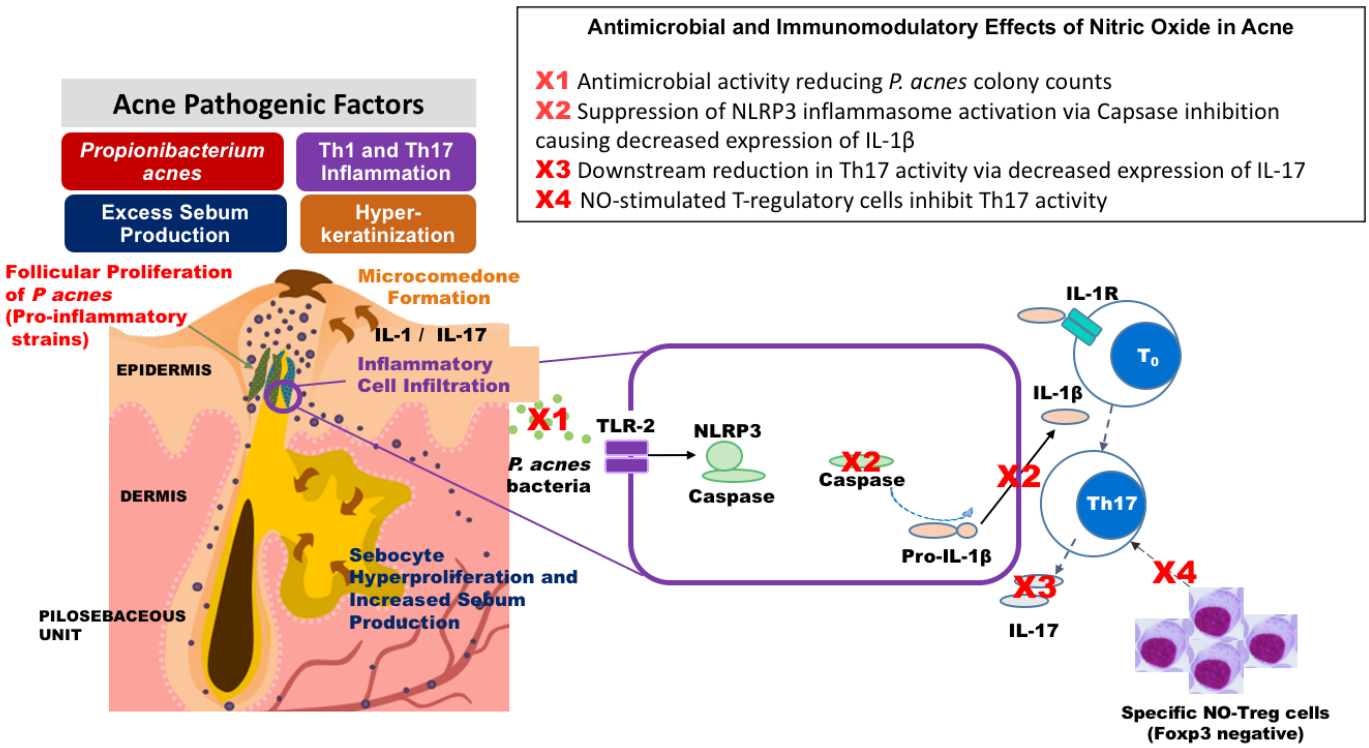
NLR – Nod-like Receptor; TGF – Transforming Growth Factor

of a topical delivery system that is effective and safe. The pharmacokinetic profile of a given NO-releasing formulation must also avoid adverse cutaneous or systemic sequelae. Several NO delivery platforms have been evaluated, many for topical application and with ongoing development programs, and each with potential advantages and disadvantages, which have been summarized in the literature; these include acidified nitrate, diazeniumdiolates (NONOates), nanoparticles, probiotic patch, S-nitrosothiols (RSNOs), and NO-metal complexes.[2,3,5,11,14,15,17,19,20] Studies completed with some NO-based topical formulations, such as with nanoparticles or with a macromolecule that incorporates N-diazeniumdiolate donor technology, provide both cogent basic science and clinical research support for the use of NO-based topical therapy for the treatment of acne.[11,14,15,17,19,38]

How Might a Nitric-Oxide Releasing Formulation Therapeutically Modify the Pathophysiology of Acne?

Understanding of the pathophysiology of acne has increased substantially over the past one to two decades due to major advancements in basic science technologies, such as immunohistochemistry, gene array analysis, and real-time polymerase chain reaction, with studies supporting the newer information on relevant pathways thoroughly summarized in the literature.[32-35] Although follicular hyperkeratinization and microcomedone formation are well known to occur during the preclinical development of an acne lesion, subclinical inflammation characterized by T-helper (CD4+) cell and macrophage infiltration, follicular production of IL-1, upregulation of vascular adhesion molecules, and a relative absence of neutrophils has also been documented.[32-35] Increased expression of IL-1 has been noted in association with comedone formation and in early inflammatory lesions and appears to play roles in both the initiation and propagation of acne.[34] These data collectively support the presence of preclinical inflammation prior to both visible inflammatory and comedonal acne lesion emergence. Importantly, persistent inflammation is also present after visible acne lesions progress from being palpable to becoming macular, with scar formation dependent on the capacity for physiologic tissue restoration within the dermal matrix.[32,34]

FIGURE 1. Acne pathophysiology and the modes of action of NO that are likely to be operative in acne treatment.



Note contribution of IL-1 during comedogenesis and progession of inflammation in acne.

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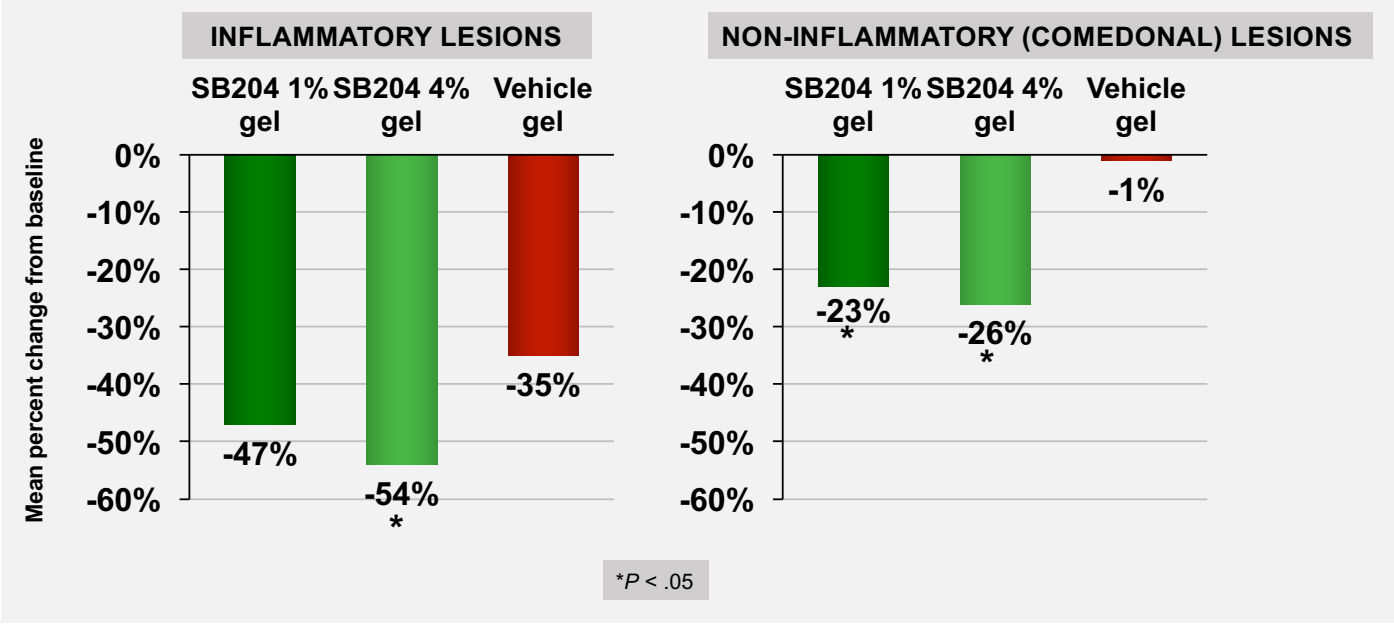
FIGURE 2. Phase 2 efficacy and safety dose-ranging study of nitric oxide (NO)-releasing topical gel in acne (N=153).# Percent change in lesion counts from baseline to week 12.
(Adapted from Baldwin H, Blanco D, McKeever C, et al. *J Clin Aesthet Dermatol.* 2016;9(8):12-18.)



Topical gel formulation utilizes an NO-releasing macromolecule composed of N-diazeniumdiolate NO donors bound covalently to a polysiloxane backbone.
SB204 NO formulation designed to administer NO similar to high flux release achieved physiologically by inducible nitric synthase (iNOS) activation.
Study Populations: SB204 4% gel (n=50), SB204 1% gel (n=51), vehicle gel (n=52); All study medications applied twice daily for 12 weeks.
Intent-to-treat population: Subjects ≥12 years of age with predominantly moderate severity acne based on Investigator Global Assessment.
Average Inflammatory Lesion Count Range, 29.0-29.3; Average Non-Inflammatory (Comedonal) Lesion Range, 49.0-51.5.
*Least squares means and *P* values from an analysis of covariance with factors of treatment group and investigational site and baseline lesion count as a covariate.

It has been noted that *P acnes* proliferation is not present during the early phase of comedone formation, and that not all strains have been associated with acne lesion development.[32,34,39] Nevertheless, available evidence supports that follicular proliferation of pro-inflammatory strains of *P acnes* contribute to acne lesion development and progression through interaction with different components of the innate immune interaction; these include the following reported mechanisms.[28-30,32-36,39]

***P acnes* Interaction With Toll-like Receptors (TLRs):** Upregulation and activation of TLRs (TLR-2, TLR-4) on inflammatory cell membranes triggers cytokine release (ie, IL-1, IL-8, IL-12, TNFα); presence of increased perifollicular staining of TLR-2 noted in acne lesions; density of TLR-2 correlated with greater acne severity.

***P acnes* Activation of the Inflammasome:** Increase in expression and activation of cytoplasmic Nod-like receptor-P3 (NLRP3) and caspase-1, which increases production of IL-1β by inflammatory cells; accentuation of comedogenesis and inflammatory lesion formation.

***P acnes* Induction of Th1 and Th17 Responses:** Increased IL-17 gene expression; increased IL-17 expression from CD4+ T lymphocytes; induction of both Th1 and Th17 immune responses in acne influence patterns of inflammatory cell infiltration and associated inflammation.

***P acnes* Activation of Antimicrobial Peptides:** Increased production of antimicrobial peptides (AMPs), such as human β-defensin-2, as response to *P acnes* propogates perifollicular inflammation; sebum free fatty acids may contribute to induced AMP expression.

***P acnes* Upregulation of Matrix Metalloproteinases (MMPs)** via transcription activator protein-1 (AP-1), which may contribute to abberant dermal matrix remodeling in acne (ie, acne scarring).

Evaluation of the biologic activities of NO, how it can inhibit pathophysiologic pathways operative in acne, and clinical study results using a specific topical NO-releasing formulation to treat acne demonstate that NO use for acne is very promising. Biologic effects of NO reported in different research studies that may correlate with inhibition of acne pathophysiology are both an *antimicrobial effect* with reduction in *P acnes*, and *multiple anti-inflammatory activities.* These anti-inflammatory modes of action are depicted in Table 2.

The ability of NO to inhibit IL-1β production and modulate Th-17 activity appear to be the most strongly supported anti-inflammatory modes of action in acne treatment based on available literature. Figure 1 summarizes acne pathophysiology and the modes of action of NO that are likely to be operative in acne treatment.

Evidence supporting the therapeutic benefit of topically applied NO has been confirmed in a Phase 2 efficacy and safety, dose-ranging, vehicle-controlled, randomized, 12-week study in subjects ≥12 years of age with mild, moderate, or severe acne. This study utilized a 1% (n=51) or a 4% (n=50) topical gel formulation (SB204), applied twice daily, of a NO-releasing macromolecule comprised of N-diazeniumdiolate NO donors covalently bound to a polysiloxane backbone.[14] This topical formulation is designed to release NO in a high-flux burst, similar to what occurs with iNOS activity. A prior pharmacokinetic (PK) study of systemic exposure to a higher concentration of the same topically applied formulation (SB204 8%) applied twice daily over 17% body surface area for 5 days showed no detectable parent compound nor increase in plasma nitrate concentrations and no alterations in hematologic indices, methemoglobin levels, or chemistry panels as compared to vehicle gel.[38]The details of the Phase 2 study efficacy and safety study are published elsewhere, and utilized recognized study methodologies including inclusion criteria, exclusion criteria, washout periods, and assessment parameters of efficacy, tolerability, and safety. Figures 2 shows that both concentrations were effective and safe, with the 4% topical NO-releasing gel significantly reducing both inflammatory and non-inflammatory (comedonal) acne lesions as compared to vehicle; tolerability and safety were also favorable with both active concentrations.[14]

What Concluding Statements Can Be Made About Nitric Oxide and Its Potential for Therapeutic Applications in Dermatology Based on the Information Discussed and Literature Review?

NO is an endogenously produced compound that exhibits several biologic properties including antimicrobial and immunolmodulatory effects that may have application for management of skin disorders, including cutaneous infections, inflammatory disorders, and wound healing. The physiologic characteristics of NO, a diatomic gas, provide challenges related to stability and delivery that require creative and novel formulation approaches.

NO application continues to be evaluated using a variety of delivery platforms, mostly topical approaches. A recent Phase 2 randomized, vehicle-controlled study of patients with acne has demonstrated efficacy and safety with twice daily application over 12 weeks of a topical NO-releasing gel that incorporates NO donors into a macromolecule formulation stabilized both for container storage and activation when released for topical application.

The major modes of action of topical NO that have been shown to inhibit acne pathophysiology are both antimicrobial (reduction in *P acnes*) and anti-inflammatory (decreased expression of IL-1β through inflammasome inhibition). Other immunoregulatory effects are also suggested as reviewed above. NO may provide a unique approach to acne treatment through inhibition of IL-1β and Th17 activation.

Microbial resistance to NO has not been demonstrated with high delivery concentrations provided with exogenous application. The antimicrobial modes of action of NO and its derivatives are diverse and cytotoxic with capability to inhibit many microbial pathogens, including several that are related to skin disorders (ie, *P acnes*, staphylococci, streptococci, dermatophytes, *C albicans*). This is very relevant clinically as avoidance of antibiotic resistance is a major issue, especially in dermatology where common skin disorders (ie, acne, rosacea) are often treated with prolonged antibiotic therapy.

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