 

# NEWS, VIEWS, & REVIEWS

# Nitric Oxide as a Promising Antiviral Agent: What Dermatologists Should kNOw

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## INTRODUCTION

Nitric oxide (NO) is an endogenous molecule produced by nitric oxide synthase (NOS) in the 2-step oxidation reaction of L-arginine.[1] NO readily diffuses and is highly reactive, causing it to have a broad range of physiologic and pathophysiologic effects. NO plays a role in crucial physiologic processes throughout the body including regulating vascular tone, neurotransmission, and immune responses.[2,3] In skin, NO is involved with maintenance and regulation of the skin barrier, antimicrobial defense, maintaining circulation, and response to UV irradiation.[1,4] Dysregulation of NO is implicated in numerous pathologies; both excess and low levels of NO may be detrimental.

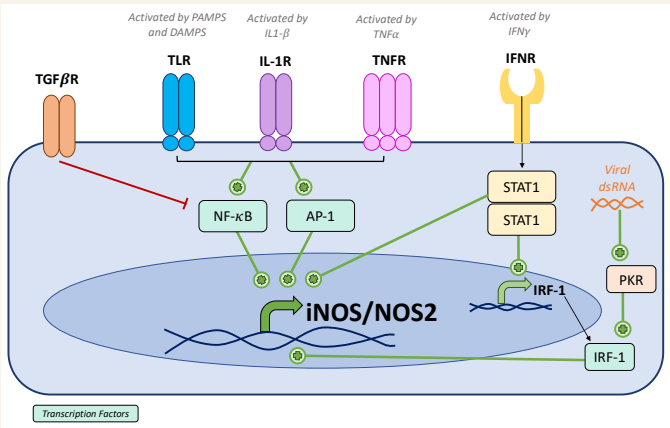
NO has immense therapeutic potential given the breadth of its interactions. Within dermatology, it has been studied most notably for its immunomodulatory properties and as a broad-spectrum antimicrobial agent with activity against bacteria, yeast, fungi, and viruses.[2,4] Herein, relevant evidence supporting the anti-viral properties of NO will be reviewed. This topic is clinically relevant for dermatologists; NO-based topical therapies are currently being explored as treatment options for viral infections, such as human papillomavirus (HPV) and molluscum contagiosum (MC).

### *Anti-Viral Properties of NO*

NO exhibits concentration-dependent immunomodulatory properties and is considered an important part of the innate immune response.[5-7] NO is produced by many immune cells including activated macrophages, dendritic cells, mast cells, natural killer cells, monocytes, eosinophils, and neutrophils.[7] At low concentrations, NO is immunostimulatory, increasing cytokine signaling, cell migration and differentiation, and vascular dilation and permeability.[4,8,9]

When viral infection occurs, there is increased transcription and activity of iNOS, an inducible isoform of NOS. iNOS transcription is multimechanistic and can be stimulated by both viral and immune factors (Figure 1).[4,6,7,10] When activated, iNOS generates a large amount of NO. At high concentrations (>1 μM), NO becomes oxidized, generating reactive nitrogen oxide species (RNOS).

**Figure 1.** Overview of iNOS/NOS2 induction pathways.[6,10]




There are multiple mechanisms by which iNOS can be stimulated. For example, toll-like receptors (TLRs) on immune and non-immune cells (ex, macrophages, lymphocytes, epithelial cells) detect pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), activating NF-κB and AP-1 signaling to upregulate iNOS. Pro-inflammatory cytokines (ex, IL1-β, TNFα) upregulate iNOS through the same pathways. Interferon gamma (IFNγ) produced by lymphocytes upregulates iNOS via the signal transducer and activator of transcription 1 (STAT-1) signaling pathway. Viral double stranded RNAs (dsRNAs) induce IFNs and bind and activate protein kinase-R (PKR), leading to upregulation of iNOS. Of note, only key signaling molecules are shown, complete pathways are not included in this figure. IRF-1= Interferon Regulatory Factor.

RNOS are important for the anti-viral and anti-microbial response.[4] RNOS nitrosylate cysteine residues of viral proteins; this process disrupts viral DNA repair enzymes and inhibits the viral replication cycle when proteases, reductases, and reverse transcriptases become inactivated.[6] RNOS also causes damage to viral DNA/RNA structure by deaminating cytosine, adenine, and guanine, inducing strand breaks, generating genotoxic alkylating agents, and causing other alterations.[4,7] With viral replication halted, virions are unable to infect additional cells, allowing for more efficient host clearance. NO can also contribute to cytotoxicity and death of infected cells by reacting with iron-containing mitochondrial enzymes, reducing their activity.[11]

Importantly, NO's genotoxic activity affects both the viral and host genome; however, host genomes contain more robust repair nucleases and polymerases.[6] Nevertheless, NO production is tightly regulated by host cells to balance indiscriminate inflammatory activity with antiviral effects; excessive NO may lead to additional complications.[6,12]

**Table 1.** Summary of Evidence for Anti-viral Activity of NO in HPV Infection.[14-17]

| Citation | Study Type | Study Purpose/Design | Results | Conclusions |
|---|---|---|---|---|
| Ormerod et al. 2015 | Phase 2, dose-finding trial<br>• Randomized<br>• Multicenter<br>• Double-blind<br>• Placebo-controlled | *Purpose:*<br>Assess treatment effect of acidified nitrite for external anogenital warts (EAW)<br><br>*Study population:*<br>299 adults with 2-50 EAW<br><br>*Treatment groups:*<br>1. Sodium nitrite 3%/citric acid 4.5% BID<br>2. Sodium nitrite 6%/citric acid 9% QD (placebo applied in AM)<br>3. Sodium nitrite 6%/citric acid 9% BID<br>4. Placebo BID<br><br>*Treatment duration:* 12 weeks<br><br>*Primary efficacy endpoint:*<br>Complete clearance of target warts | Patients who achieved complete clearance:<br>1. SN 3%/CA 4.5% BID: 15%<br>2. SN 6%/CA 9% QD: 23%<br>3. SN 6%/CA 9% BID: 31%<br>4. Placebo: 14%<br><br>Treatment site reactions in 66-92% of active treatment groups (most commonly itching) | Sodium nitrite 6%/citric acid 9% BID was more effective than placebo for treatment of anogenital warts |
| Tyring et al. 2018 | Phase 2 dose-escalation trial<br>• Randomized<br>• Double-blind<br>• Vehicle-controlled | *Purpose:*<br>Assess treatment effect of SB206 for extragenital/perianal warts (EGW/PAW)<br><br>*Study population:*<br>108 adults with 2-20 EGW/PAW<br><br>*Treatment groups:*<br>1. SB206 4% QD or BID<br>2. SB206 8% QD<br>3. SB206 12% QD<br>4. Vehicle<br><br>*Treatment duration:* 12 weeks<br><br>*Primary efficacy endpoint:*<br>Complete clearance of baseline EGW/PAW | Complete clearance was achieved in:<br>1. SB206 4% QD: 20.8%<br>2. SB206 8% QD: 14.3%<br>3. SB206 12% QD: 33.3%<br>4. Vehicle: 4.3% | Complete clearance was achieved in a higher proportion of patients in the SB206 group compared to vehicle, especially for SB206 12% QD |
| Yu et al. 2018 | In vitro study | *Purpose:*<br>Investigate role of NO in regulating HPV gene transcription<br><br>*Methods:*<br>Human cervical carcinoma cells (HPV16+) were treated with NO-donor (DETA-NO) at varying concentrations, E6 gene expression was measured by real-time PCR | DETA-NO inhibited cervical carcinoma cell proliferation and levels of HPV E6 mRNA in dose and time dependent manner | Expression of HPV E6 protein mRNA was inhibited by NO |
| Banerjee et al. 2019 | In vitro study | *Purpose:*<br>Investigate impact of exposing HPV-18 infected raft cultures to NO donor SB206<br><br>*Methods:*<br>• Primary human keratinocytes infected with HPV-18 were exposed to SB206 at various concentrations<br>• S-phase cells, E6 and E7 protein levels, HPV-18 DNA replication were assessed | SB206-treated cells compared to control had:<br>• Reduced HPV-18 DNA by 95%<br>• Decreased number of cells in S phase<br>• Decreased E6 and E7 protein levels, increased p53 protein | SB206 inhibited HPV DNA replication by reduction of E6 and E7 oncoproteins, impairing S-phase progression |

*NO-based Anti-viral Therapies*

NO has promising therapeutic applications, including as an anti-viral agent. NO has been studied in several viruses clinically relevant to dermatologists including herpes simplex virus (HSV), HPV, and MC. HSV was one of the first viruses where NO was demonstrated to have anti-viral activity; a 1993 in vitro study demonstrated that NO reduced HSV1 replication, protein, and DNA synthesis in macrophages in vitro, and addition of a NOS inhibitor reduced the anti-viral effect of macrophages.[13]

In HPV and MC, NO is actively being studied in vivo and in vitro as a potential treatment for infection. NO has been shown in vitro to inhibit HPV DNA replication through reduction of E6 and E7 oncoproteins and has demonstrated success in treating anogenital warts in clinical trials (Table 1).[14-17] Efficacy of NO for MC infection was first seen in a 1999 clinical trial: a nitric oxide donor coadministered with 5% salicylic acid under occlusion was more effective than salicylic acid alone in treating MC (cure rate 75% vs 21%), however, the tested formulation caused frequent

**Table 2.** Summary of Evidence from Clinical Trials of SB206 for MC Infection.[20-23]

| Citation | Trial Type | Trial Purpose/Design | Results | Conclusions |
|---|---|---|---|---|
| Hebert et al. 2020 | Phase 2, dose-finding trial<br>• Randomized<br>• Multicenter<br>• Double-blind<br>• Vehicle-controlled | *Purpose*:<br>Assess treatment effect of SB206 for MC lesions<br><br>*Study population:*<br>256 patients (age ≥2 YO) with MC lesions<br>• Mean baseline lesions=18.3 (vehicle), 19.3 (SB206)<br><br>*Treatment groups:*<br>1. SB206 4% BID<br>2. SB206 8% BID<br>3. SB206 12% QD or BID<br>4. Vehicle<br><br>*Treatment duration:* 12 weeks<br><br>*Primary efficacy endpoint:*<br>Complete clearance of MC lesions | Patients who achieved complete clearance:<br>1. SB206 4% BID: 10.6%<br>2. SB206 8% BID: 33.3%<br>3. SB2016 12% BID: 27.7%<br>4. SB206 12% QD: 37.5%<br>5. Vehicle: 18.2%<br><br>40-50% reported mild-moderate AEs in treatment groups | SB206 12% QD dose had greatest MC lesion clearance |
| Maeda-Chubachi et al. 2021 | Integrated analysis of 2 Phase 3 clinical trials (NCT03927703, NCT03927716)<br>• Randomized<br>• Multicenter<br>• Double-blind<br>• Vehicle-controlled | *Purpose*:<br>Assess impact of SB206 on BOTE* status, and BOTE status on MC lesion reduction<br><br>*Study population:*<br>707 patients (age ≥6 mo) with MC lesions<br>• Mean baseline lesions=17.8 (vehicle), 18.4 (SB206)<br>• Baseline BOTE Status: 34.8% BOTE+, 64.4% BOTE-<br><br>*Treatment groups:*<br>1. SB206 12% QD<br>2. Vehicle<br><br>*Treatment duration:* 12 weeks<br><br>*Outcomes evaluated:*<br>BOTE score over time, BOTE score and MC lesion reduction | • 80% incidence of BOTE sign, regardless of treatment assignment<br><br>• At week 12, MC lesion count decreased from baseline by:<br>1. *SB206:* 63.3% for BOTE+, 51.7% for BOTE-; p=0.0194<br>2. *Vehicle:* 50.7% for BOTE+, 29.1% for BOTE-; p=0.0015<br><br>• Baseline BOTE+ patients treated with SB206 had overall greatest lesion reduction over time<br><br>Most common AEs were application-site pain and erythema | Patients who were both BOTE+ and treated with SB206 had the greatest reduction in MC lesion count<br><br>SB206 may trigger BOTE sign, promote faster lesion clearance |
| Cartwright et al. 2022 | Phase 1 prospective, open-label study<br>• Multicenter | *Purpose:*<br>Evaluate safety, tolerability, pharmacokinetic parameters of SB206 10.3%<br><br>*Study population:*<br>34 patients with (Age ≥2YO) with 20+ MC lesions<br>• Mean baseline lesions=50<br>• Total treatment area= 484 $cm^2$<br><br>*Treatment:* SB206 10.3% QD<br><br>*Treatment duration:*<br>• 2 week pharmacokinetic period<br>• 10 week treatment extension | • Minimal systemic exposure of SB206<br>• Progressive decrease in baseline MC lesions was seen<br>• 4 patients achieved complete clearance at week 12<br><br>Mild-moderate AEs reported in 47% of treatment group, most commonly application site erythema or pain | SB206 10.3% gel applied QD was well-tolerated with minimal systemic absorption |
| Browning et al. 2022 | Phase 3 clinical trial<br>• Randomized<br>• Multicenter<br>• Double-blind<br>• Vehicle-controlled | *Study population:*<br>891 patients (age ≥6 mo) with 3-70 MC lesions<br>• Mean baseline lesions=20.5 (vehicle), 23.1 (SB206)<br><br>*Treatment groups:*<br>1. SB206 10.3% gel QD<br>2.Vehicle<br><br>*Treatment duration:* 12 weeks<br><br>*Primary efficacy endpoint:*<br>% difference of patients who achieve complete clearance of MC lesions | • Patients who achieved complete clearance of all MC lesions:<br>1. SB206: 32.4%<br>2. Vehicle: 19.7%<br>*Absolute difference: 12.7%, P<0.001*<br><br>• Patients who achieved 90%+ reduction in baseline lesion count:<br>1. SB206: 43%<br>2. Vehicle: 23.9%<br><br>Mild-moderate AEs reported in 43% of treatment group, most commonly application site erythema or pain | Treatment with SB206 10.3% gel for 12 weeks resulted in significantly greater complete MC lesion clearance than patients treated with vehicle |

* Beginning of the end (BOTE) sign refers to clinical inflammatory signs that predict imminent resolution of MC. BOTE+ indicates presence of BOTE sign, while BOTE- indicates that it is not present.

side effects of skin staining and irritation.[18] More recently, SB206, a NO-releasing topical medication, has shown encouraging results for treatment of MC in clinical trials. SB206 is comprised of a gel containing berdazimer sodium, a macromolecule covalently bound to NO donors, and a hydrogel that acts as a proton donor.[19] Evidence from the clinical trials of SB206 in MC can be found in Table 2. In 2023, SB206 was submitted to the US Food and Drug Administration as a New Drug Application; if accepted, this would be the first approved therapy for MC.

## Conclusion

NO has therapeutic potential as an anti-viral agent. The observations from in vitro and in vivo work to date suggest that NO-releasing therapies should be further developed, tested, and explored in viral infections, such as HPV and MC. Future comparative trials will be required to assess efficacy of SB206 and other NO-based therapies relative to currently available treatments.

## Disclosure

EM and SD have no relevant conflicts to disclose. AF has developed several nitric-oxide releasing technologies, though none are referenced in this paper.

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