Topical Nitric Oxide as a First-in-Class, Local Antiandrogen Therapy for the Treatment of Acne and Male Pattern Baldness

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Introduction

Novan Therapeutics, a clinical-stage drug-development company, has developed drug candidate SB204, which releases nitric oxide when applied topically to the skin. Based on results from animal studies, locally available nitric oxide may inhibit skin steroidogenesis resulting in reduced levels of androgens like testosterone and 5α-dihydrotestosterone (5α-DHT). Androgens in skin are thought to stimulate oil production that drives the development of acne as well as promote hair loss in men. By reducing androgen levels locally in skin, topical nitric oxide may lead to first-in-class local antiandrogen therapies used to treat these disease processes.

This white paper will discuss the role of androgens in these disease processes and provide an overview of the mechanistic literature supporting nitric oxide as a local antiandrogen therapy. The following topics will be discussed:

- Introduction to systemic androgens and steroid hormone biosynthesis
- Androgen production in the skin
- Therapeutic benefit of antiandrogen therapy for the treatment of acne
- Therapeutic benefit of antiandrogen therapy for the treatment of androgenetic alopecia (male pattern baldness)
- Summary of mechanisms by which nitric oxide inhibits androgen and lipid synthesis in the skin
- Hamster flank data supporting the antiandrogenic activity of SB204
- Rodent and minipig pharmacokinetics demonstrating no systemic bioavailability of nitric oxide after topical application of SB204

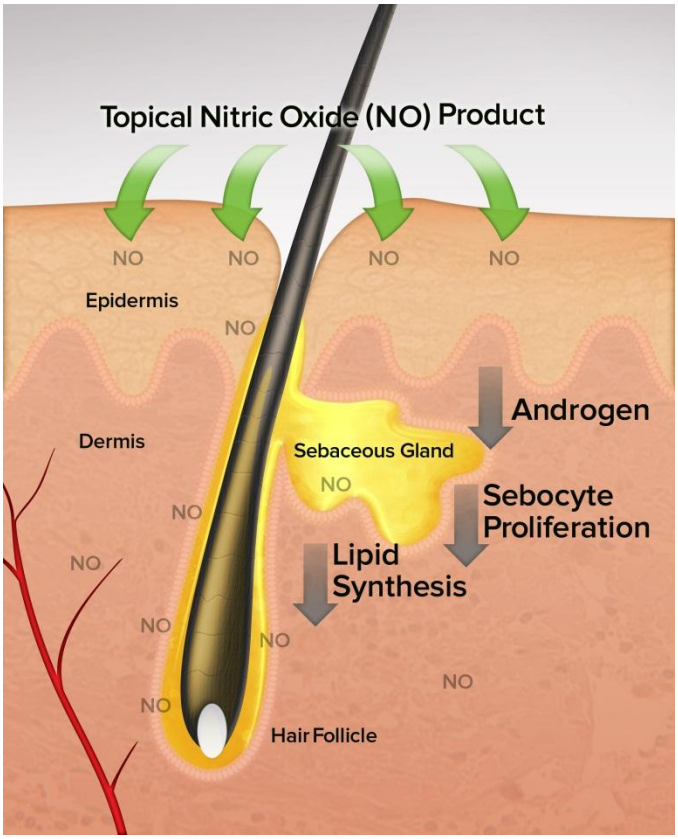

Figure 1: *Nitric Oxide Diffusion through the Skin and Potential Localized Effects of Nitric Oxide on Decreasing Androgen Production as well as Decreasing Sebum Production, the Oily Secretions of the Sebaceous Gland.*

Steroid Hormone Biosynthesis

Androgens are steroid hormones produced and secreted into the systemic circulation by the testes, ovaries, and adrenal gland. Androgens acting through their cognate receptors direct the development of male reproductive tissues during fetal and pubertal development and promote the development of male sexual characteristics such as increased muscle and bone mass, the growth of body hair, deepening of the voice, and sexual arousal. As hormones, androgens are produced in specific regions of the body and are distributed by the circulatory system to act in other parts of the body (i.e., in tissues like the skin that contain receptors for androgens).

Like other steroid hormones, androgens are derived from cholesterol, intracellular levels of which are controlled by sterol regulatory element binding protein (SREBP-1). An initial step in androgen biosynthesis is the oxidative cleavage of the side chain of cholesterol by the mitochondrial cholesterol side-chain cleavage enzyme (CYP11A), to yield pregnenolone. Pregnenolone is converted to testosterone through the action of 3β-hydroxysteroid dehydrogenase/isomerase (3β-HSD), 17α-hydroxylase/C_{17-20}-lyase (CYP17A1) and 17β-hydroxysteroid dehydrogenase (17β-HSD). Testosterone can then be further reduced to the more potent androgen, 5α-DHT, by 5α-reductase.

Enzymes like cytochrome P450 enzymes and 5α-reductase are susceptible to inhibition by nitric oxide due to specific structural groups contained in their active sites that are critical for enzyme activity.

Androgen Biosynthesis in the Skin

The skin can synthesize androgens *de novo* from cholesterol or by locally converting circulating weaker androgens, like dehydroepi-androsterone (DHEA) or its sulfated derivative (DHEA-S), into more potent androgens like testosterone and 5α-DHT (Chen 2002; Slominsky 2013). The skin contains steroidogenic acute regulatory protein (StAR), CYP11A, 3β-HSD, CYP17A1, 17β-HSD, 5α-reductase (5αR1/2) and many other steroidogenic enzymes serving to define the skin as an independent steroidogenic organ (Slominski 2013). The local production of androgens in the skin can be endogenously regulated by locally produced hormones like corticotropin-releasing factor (CRF), adrenocorticotropic hormone (ACTH), or by cytokines. In fact, skin from both acne (Sansone 1971) and male pattern baldness (Puerto 1990) patients produces higher rates and amounts of testosterone and 5α-DHT than skin from healthy individuals (Fritsch 2001).

Acne and Androgens

Acne vulgaris is the most common disorder involving the sebaceous gland and the most common skin disease in the United States. Forty to fifty million Americans have acne, including up to 80% of adolescents (Smith and Thiboutot 2008).

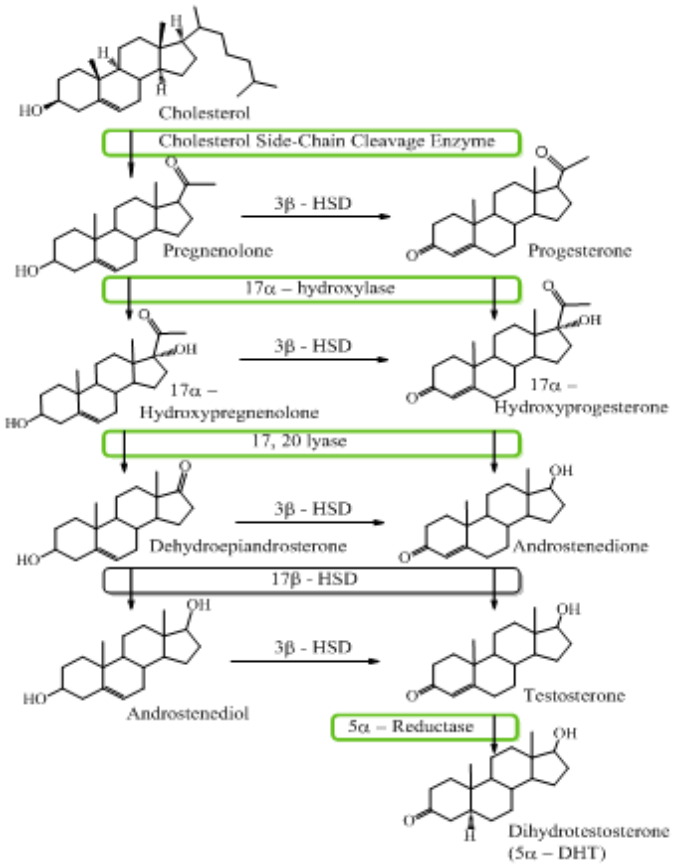

Figure 2: *Biosynthetic Pathways for Androgen Synthesis. The key enzyme activities (e.g., CYP11A, CYP17A1 and 5αR1/2) susceptible to inhibition by nitric oxide (i.e., heme- or tyrosine-containing critical residues) are highlighted in green. The hypothesized 5α-reductase inhibitory activity of nitric oxide can be exploited for local therapeutic benefit of acne and androgenetic alopecia.*

Acne can cause both physical and psychological effects, including anxiety, depression and poor self-esteem (Thiboutot 2000). Acne lesions develop when excess sebum and abnormal epithelial desquamation blocks the opening of a follicle, forming a microcomedo. The anaerobic, lipid-rich environment of the microcomedo is ideal for *P. acnes* proliferation. Since androgens exert their effects on sebaceous glands by increasing sebocyte proliferation and increasing total lipid synthesis, inhibition of androgen production by or action in the skin should reduce sebum production and *P. acnes* proliferation, and consequently be effective for the treatment of acne. This hypothesis is confirmed by the fact that patients with complete androgen insensitivity do not produce sebum and do not develop acne (Imperato-McGinley 1993).

Castrated males produce minimal sebum and also do not develop acne, a situation which reverses when treatment with testosterone is initiated (Imperato-McGinley 1993).

Androgenetic Alopecia and Androgens

Androgenetic alopecia or "male pattern baldness" is the most common form of inherited hair loss affecting up to 50% of the Caucasian male population by middle age (Lai 2012). The development of androgenetic alopecia depends on the individual's genetic predisposition, and the presence of sufficient androgens, androgen receptors, and androgen receptor coactivators in the skin. Balding is NOT observed in individuals that lack a functional androgen receptor (Griffin and Wilson 1989), in men castrated at puberty (Hamilton 1942), or in men with 5α-reductase deficiency, the enzyme that converts testosterone to the more potent androgen, 5α-DHT (Inui 2012).

Inhibition of androgen production by or action in the skin is expected to be effective in treating male pattern baldness, as evidenced by the use of the 5α-reductase inhibitor finasteride (Propecia®) to treat this disease process (Alsantali and Shapiro 2009).

Androgens like 5α-DHT accelerate the rate at which hair follicle miniaturization takes place, i.e., as new hair follicles are generated they are smaller than their predecessors eventually leading to miniaturized follicles that do not support hair growth (Garza 2012). Another recent mechanistic player in this field includes prostaglandin D2. Diminished conversion of hair follicle stem cells to progenitor cells is critical in the pathogenesis of male pattern baldness along with expression of the prostaglandin D2 synthase gene and PGD2 levels, both of which are dramatically increased in hair follicle biopsies taken from balding individuals (Garza 2011). Whether PGD2 is the signal that inhibits stem cell to progenitor cell conversion is not understood at this time, however, knockout animals made null for the PGD2 (DP-2) receptor suggest this may be the case (Garza 2012).

The immediate precursor for the synthesis of PGD2 is the cytochrome P450-dependent conversion of arachidonic acid to PGH2 by cyclooxygenase, an enzymatic activity known to be inhibited by nitric oxide via tyrosine nitration (Goodwin 1998; Goodwin 1999; Deeb 2006).

By inhibiting androgen and PGD2 synthesis nitric oxide may have multiple mechanisms that can be exploited to treat male pattern baldness.

Summary of mechanisms by which nitric oxide can inhibit androgen and lipid synthesis in the skin

Cytochrome P450 enzymes play a critical role in the metabolism of physiological substrates such as steroids, fatty acids, prostaglandins, and environmental xenobiotics. Nitric oxide reacts with various molecules including superoxide, iron, thiol compounds, tyrosine and various hemoproteins including P450 (Minamiyama 1997). Nitric oxide reacts reversibly with the heme iron of P450 forming iron-nitrosyl complexes and irreversibly by nitration of tyrosine residues or oxidation of critical thiol residues requisite for enzyme activity (Minamiyama 1997; Morgan 2001). In the steroid biosynthetic pathway, nitric oxide inhibits heme-containing P450 enzymes, namely, cholesterol side-chain cleavage enzyme (CYP11A1; Drewett 2002; Del Punta 1996), 17α-hydroxylase/C_{17-20}-lyase (CYP17A1; Pomerantz and Pitelka 1998), and aromatase (CYP19A1; Snyder 1996), presumably by interacting with heme iron of P450 (Figure 2). Since *de novo* androgen production in the skin requires CYP11A1 and CYP17A1, inhibition of these enzymes could reduce skin androgen levels thus reducing sebocyte proliferation, decreasing total lipid synthesis, sebum production, and subsequent *P. acnes* proliferation for the treatment of acne (Figure 1).

Alternatively, or in addition, nitric oxide may inhibit the activity of 5α-reductase, the enzyme that converts testosterone to the more potent androgen 5α-DHT. A critical and highly conserved catalytic residue (tyrosine^{58}) in the active site of the 5β-reductase enzyme in liver (Costanzo 2008) may be susceptible to nitrosation and inactivation by nitric oxide. Studies are underway to determine the ability of nitric oxide to inhibit 5α-reductase enzymatic activity in vitro. Finally, nitric oxide may regulate lipid synthesis directly via nitrosative stress, covalently binding to coenzyme A (CoA), and

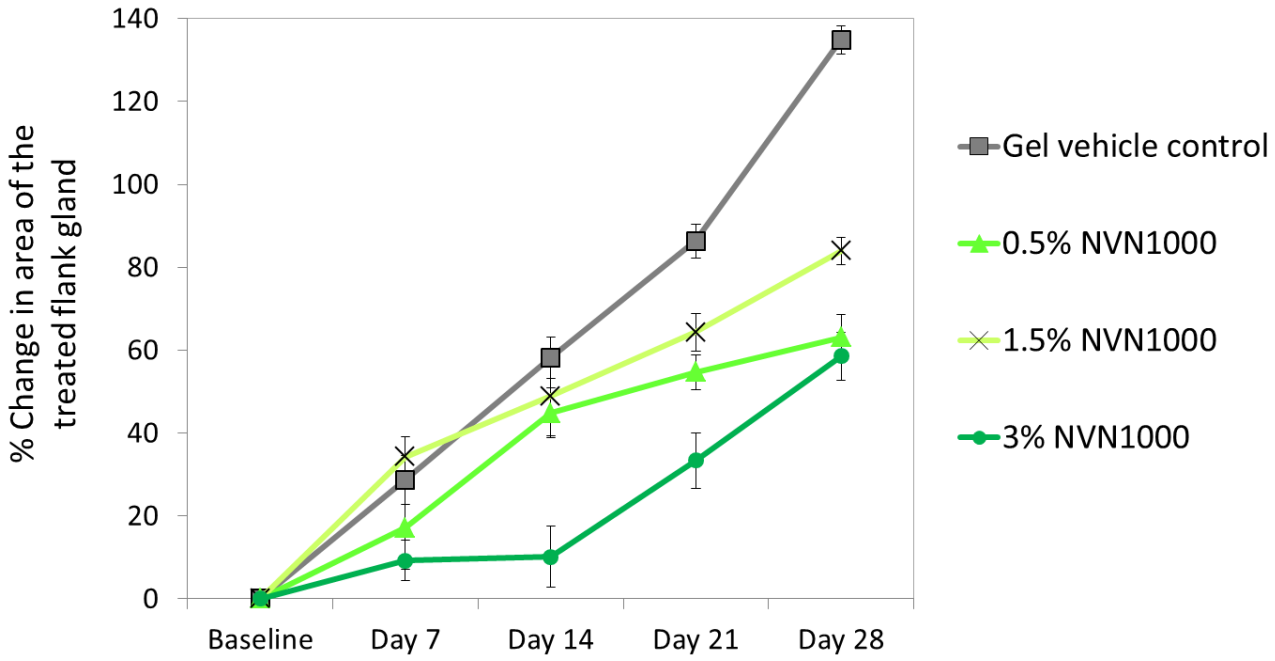

Figure 3: *Dose-Response of Topically Applied SB204 (NVN1000 Hydroalcoholic Gel) on the Percent Change in Area of the Treated Flank Gland Area in Pubertal Male Syrian Hamsters. (Error bars reported as SEM)*

producing metabolically inactive S-nitrosoCoA, which is central to the pathway of lipid and cholesterol synthesis (Roediger 2004).

Hamster flank organ data supporting the topical antiandrogen activity of SB204

Male Golden Syrian hamsters have paired pelvic flank glands which grow in size in response to androgens during pubertal development. The androgen-sensitive components of the flank organ include dermal melanocytes, sebaceous glands, and hair follicles. This model is routinely used to evaluate the anti-androgenic activity of topically applied substances. As androgens stimulate the sebaceous gland to produce sebum, anti-androgen activity in the hamster flank gland model may predict the ability of SB204 to reduce sebum production and to treat or prevent the development of acne.

To investigate this hypothesis, the ability of topically applied SB204 (0.5, 1.5, and 3% SB204) to suppress androgen-mediated growth of the flank gland in male Golden Syrian hamsters was investigated (Figure 3). Vehicle control treatments were included for comparison. The treatments were applied topically once daily for 28 days to the right flank gland. The left gland was not treated, and used as an internal control for systemic anti-androgenic activity (or lack thereof). The gland areas were measured on Day 0, 7, 14, 21, and 28. Bodyweights were recorded twice weekly.

SB204 significantly inhibited the growth of androgen-dependent hamster flank glands. Inhibition occurred faster at higher doses and was first evident at 14 days at the highest dose. After 28 days of dosing, all doses significantly inhibited flank gland growth compared to the gel vehicle control group (Figure 3).

There was no evidence of systemic absorption/activity, as left flank glands in SB204-treated groups were not significantly different in size compared to flank glands in the gel vehicle control group. This is consistent with all pharmacokinetic evaluations completed to date with SB204 (see below). No signs of systemic toxicity were observed. Additionally, all animals gained weight over the course of the study and no animals exhibited any adverse effects.

Topically applied SB204 exhibits no systemic bioavailability

While nitric oxide released from SB204 is anticipated to be pharmacologically active in the

skin, it is expected to be extremely short-lived (milliseconds) and local. Nitrate is quantitatively the most important end product of nitric oxide metabolism after subcutaneous nitric oxide administration (Benthin 1997); hence nitrate levels were measured in nonclinical studies to assess the potential for systemic bioavailability. Maximum feasible doses were assessed (maximum feasible concentration and maximum feasible volume applied over 10% of the total body surface area). Nonclinical studies performed to date have not shown any safety concerns or appreciable systemic exposure when applied topically to rodents and minipigs for as long as 28-days in repeat-dose studies. These results indicate that nitric oxide therapies applied topically may induce only local – and not systemic – effects on steroidogenesis and lipid biosynthesis, suggesting SB204 has the potential to be a first-in-class local antiandrogen.

Novan Therapeutics is a development stage company subject to the risks and uncertainties associated with product development.

About the Author

Dr. William R. Kelce
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Novan Therapeutics

Dr. Kelce is internationally recognized as an expert in endocrine toxicology including inhibitors of androgen synthesis and inhibitors of androgen receptor action. Dr. Kelce, along with collaborators at the University of North Carolina-Chapel Hill and the US Environmental Protection Agency, were the first to detect anti-androgen substances in the environment and elucidate their biochemical and molecular mechanisms of action (Kelce 1995; Wong 1995). Dr. Kelce serves on several peer review editorial boards, is Associate Editor for Toxicology and Applied Pharmacology, a Pharmacia Science Fellow, a Fellow of the Academy of Toxicological Sciences, and a member of the Society of Toxicology, the American College of Toxicology, and numerous other scientific organizations

Literature Citations

Alsantali, A. et al. Androgens and hair loss. *Curr. Opin. Endocrinol. Diabetes Obes.* **2009,** *16,* 246-253.

Benthin, G. et al. Transformation of subcutaneous nitric oxide into nitrate in the rat. *Biochem. J.* **1997,** *323, 853-858.*

Chen, W. et al. Cutaneous androgen metabolism: basic research and clinical perspectives. *J. Invest. Dermatol.* **2002**, *119,* 992-1007.

Deeb, R. et al. Inducible nitric oxide synthase mediates prostaglandin H_2 synthase nitration and suppresses eicosanoid production. *Am. J. Pathol.* **2006,** *168,* 349-362.

Del Punta, K. et al. Nitric oxide inhibits Leydig cell steroidogenesis. *Endocrinology*. **1996**, *137*, 5337-5343.

Di Costanzo, L. et al. Crystal structure of human liver Δ^4-3-ketosteroid 5β-reductase (AKR1D1) and implications for substrate binding and catalysis. *J. Biol. Chem.* **2008,** *283,* 16830-16839.

Drewett, J. et al. Nitric oxide potently inhibits the rate-limiting enzymatic step in steroidogenesis. *Mol. Cell. Endocrinol.* **2002**, *194,* 39-50.

Fritsch, M. et al. Sebocytes are the key regulators of androgen homeostasis in human skin.. *J Invest. Dermatol.* **2001,** *116*, 793-800.

Garza, L. et al. Prostaglandin D_2 inhibits hair growth and is elevated in bald scalp of men with androgenic alopecia. *Sci. Transl. Med.* **2012**, *4,* 1-11.

Garza, L. et al. Bald scalp in men with androgenic alopecia retains hair follicle stem cells but lacks CD200-rich and CD34-positive hair follicle progenitor cells. *J. Clin. Invest.* **2011**, *121,* 613-622.

Griffin, J. et al. In *The metabolic basis of inherited disease.* Scriver, C. et al. Eds. 1989. McGraw-Hill, New York, 1919-1944.

Goodwin, D. et al. Nitric oxide trapping of tyrosyl radicals generated during prostaglandin endoperoxide synthase turnover. *J. Biol. Chem.* **1998**, *273*, 8903-8909.

Goodwin, D. et al. Effects of nitric oxide and nitric oxide-derived species on prostaglandin endoperoxide synthase and prostaglandin biosynthesis. *FASEB J.* **1999,** *13,* 1121-1136.

Hamilton, J. et al. Male hormone stimulation is prerequisite and an incitant in common baldness. *Am. J. Anat.* **1942,** 71, 451-480.

Imperato-McGinley, J. et al. The androgen control of sebum production. Studies of subjects with dihydrotestosterone deficiency and complete androgen insensitivity. *J. Clin. Endocrinol. Metab.* **1993,** *76*, 524-528.

Inui, S. et al. Androgen actions on the human hair follicle: perspectives. *Exp. Dermatol.* **2012,** *22,* 168-171.

Kelce, W. et al. Persistent DDT Metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature*. **1995,** *375,* 581-585.

Lai, J. et al. The role of androgen and androgen receptor in skin-related disorders. *Arch. Dermatol. Res.* **2012**, *304*, 499-510.

Minamiyama, Y. et al. Irreversible inhibition of cytochrome P450 by nitric oxide. **1997,** *283*, 1479-1485.

Morgan, E. et al. Cytochromes P450 and flavin monoozygenases – targets and sources of nitric oxide. *Drug Metab. Dispos.* **2001**, *29*, 1366-1376.

Pomerantz, D. et al. Nitric oxide is a mediator of the inhibitory effect of activated macrophages on production of androgen by the Leydig cell of the mouse. *Endocrinology.* **1998**, *139*, 922-931.

Puerto, et al. Regional differences of the androgenic metabolic pattern in subjects affected by male pattern baldness. *Rev. Esp. Fisiol.* **1990,** 46, 289-296.

Roediger, W. et al. Inhibition of hepatocyte lipogenesis by nitric oxide donor: could nitric oxide regulate lipid synthesis? *IUBMB Life.* **2004,** *56*, 35-40.

Sansone, G.et al. Differential rates of conversion of testosterone to dihydrotestosterone in acne and in normal human skin – a possible pathogenic factor in acne. *J Invest. Dermatol.* **1971,** *56*, 366-372.

Slominski, A. et al. Steroidogenesis in the skin: implications for local immune functions. *J. Steroid. Biochem. Mol. Biol.* **2013,** http://dx.doi.org/10.1016/j.jsbmb.2013.02.006

Snyder, G. et al. Nitric oxide inhibits aromatase activity: mechanisms of action. *J. Steroid. Biochem.* **1996,** *56,* 63-69.

Smith, K. et al. Sebaceous glands: friend or foe? *J. Lipid Res.* **2008,** *49,* 271-281.

Thiboutot, D. New treatments and therapeutics strategies for acne. *Arch. Fam. Med.* **2000,** *9,* 179-187.

Wong, C. et al. Androgen receptor antagonist versus agonist activities of the fungicide vinclozolin relative to hydroxyflutamide. *J. Biol. Chem.* **1995,** *270,* 19998-20003.