Review Article

# Nitric oxide may directly induce the psoriatic disease process via suppression of keratinocyte apoptosis and induction of keratinocyte hyperproliferation

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**Abstract** Nitric oxide (NO) has been supposed to induce the psoriatic disease process indirectly through increase of release and also effects of substance P and calcitonin gene-related peptide. This paper discusses the potential direct roles of NO by induction of keratinocyte hyperproliferation and suppression of keratinocyte apoptosis.

NO has been shown to exert a biphasic effect on keratinocytes based on its concentration: increasing proliferation and decreasing differentiation of keratinocytes at low concentrations but producing the reverse effects at high concentrations (≥500 μm). Therefore, NO, having a maximum concentration of about 0.01 μm in the psoriatic lesions, more likely induces keratinocyte hyperproliferation rather than suppressing it.

Low production of NO in psoriasis occurs in the face of high overexpression of inducible nitric oxide synthase (iNOS) mRNA and protein and may be due to a) overexpression of arginase 1, which regulates iNOS activity by competing for the common substrate L-arginine, b) overexpression of calcitonin gene-related peptide, which inhibits iNOS activity, c) NO's regulatory effect on its own production by binding to heme which mediates iNOS dimerization, and d) NO's inhibition of the release of nerve growth factor which in synergy with TNF-α induces iNOS. Noteworthy, neopterin and cutaneous polyamines, which are also overexpressed in the psoriatic lesions, contribute further to the low production of NO via suppression of the expression of iNOS gene. Based on the observations that psoriatic keratinocytes are resistant to the induction of apoptosis and that NO, at low concentrations, is capable of suppressing apoptosis, this author suggests the apoptosis-suppressant effect of NO as another potential role for NO in inducing the psoriatic disease process.

***Key words*** Apoptosis, calcitonin gene-related peptide, keratinocyte, nitric oxide, neopterin, nerve growth factor, psoriasis.

An important development in the understanding of the pathogenesis of psoriasis has been the discovery that psoriatic plaques actively produce nitric oxide (NO).

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Kolb–Bachofen *et al.*[1] first showed that epidermal keratinocytes in psoriatic plaques produce the enzyme "inducible nitric oxide synthase (iNOS)", and Weller *et al.*[2] showed that NO synthesis is indeed increased in psoriasis. The production of NO is about 100 times higher in psoriatic plaques than the skin of normal persons.[3] These observations are highly suggestive of a role for NO in the development of psoriasis.

Giustizieri and coworkers[4] have shown that nitric oxide donors suppress chemokine production by keratinocytes *in vitro* and *in vivo*. This work as well as the finding that NO farvors a Th2 over a Th1 response, while psoriasis is characterized by a Th1–type cytokine pattern,[5] may lead to the proposal that NO exerts regulatory effects on psoriatic disease process, rather than producing it. However, it has been shown that NO exerts a biphasic effect on keratinocytes based on its concentration: increasing proliferation and decreasing differentiation of keratinocytes at low concentrations but producing the reverse effects at high concentrations (= 500 μM).[6]

It is estimated that in the psoriatic lesions, NO concentrations are about 0.00005 μM near the skin surface and increase to a maximum of about 0.01 μM in the middle of the rete pegs.[7] Taken together, though it is still difficult to reconcile the paradoxical effects of NO on keratinocytes and on the immune system, it seems more likely that NO, in concentrations produced in psoriasis, induces keratinocyte hyperproliferation rather than inhibiting it. Moreover, since high concentrations of NO are keratinocytostatic, it is not astonishing that both NO scavengers and NO donors be of value in the treatment of psoriasis. The efficacy of anthralin in treating psoriasis could therefore be explained partly by its induction of iNOS.[8] Conclusively, though NO concentration is elevated in psoriasis, it is still too low to exert antiproliferative effects, rather, it induces keratinocyte hyperproliferation.

Inducible NOS (iNOS) mRNA and protein are highly overexpressed in psoriatic lesions, however, arginase 1, which participates in the regulation of iNOS activity by competing for the common substrate L-arginine, is also overexpressed in the lesions, being co-expressed with iNOS.[9] Furthermore, calcitonin gene-related peptide (CGRP), which is overexpressed in the psoriatic lesions,[10] is capable of inhibition of iNOS activity and therefore decreasing NO concentration.[11,12] Given that NO is known to increase the release of CGRP,[10] NO could be supposed to control its own production indirectly through effecting the overexpression of CGRP. The dimerization of iNOS, mediated by the binding of iron protoporphyrin IX (heme) to the oxygenase domain of iNOS, is essential for its activation. Since NO binding to heme renders it insoluble, the iron released from the oxygenase domain prevents dimerization of iNOS proteins and uncouples already formed iNOS dimers. NO may thus directly regulate its own production.[11] Nerve growth factor (NGF), which is overexprssed in the psoriatic lesions,[13] is known to induce iNOS in synergy with tumor necrosis factor-alpha (TNF-α),[14] which is also overexpressed in the lesions. NO is known to inhibit the basal nerve growth factor release through increase of cGMP levels[16] and hence indirectly controls its own production. Neopterin, a compound belonging to the unconjugated pteridines group, is mainly synthesized by monocytes/macrophages upon stimulation with Th1-deriven IFN-gamma. Neopterin has been proposed to inhibit the NO-induced apoptotic cell death via suppression of cytokine-induced NO synthesis, say, via suppressing the iNOS gene expression.[17] Therefore, neopterin has been supposed to encourage tumor cell growth and proliferation.[17,18] It has been demostrated that

serum and urinary neopterin levels are elevated in psoriatics.[19] Thus, neopterin may also be partly responsible for the low NO concentration in the psoriatic lesions. Moreover, cutaneous polyamines, which are known to be increased in psoriasis,[20] further decrease the concentration of NO through suppression of iNOS gene expression.[21]

Morhenn[10] proposed that NO could trigger keratinocyte hyperproliferation through stimulation of guanylate cyclase in the keratinocytes, thereby producing cyclic guanosine monophosphate (cGMP) nucleotide which is supposed to be a potential mitogen for keratinocytes. However, this view was opposed by some workers, as some studies suggest that cGMP arrests keratinocyte proliferation and promotes differentiation, possibly by cGMP-gated $Ca^{+2}$ channel activation. Therefore, no mechanism has been established for NO-induced keratinocyte hyperproliferation until very recently; that it has been proposed that NO may induce the psoriatic disease process indirectly through its ability in augmenting both the release and effects of CGRP and substance P, which are considered to play important roles in the pathomechanism of psoriasis.[10] The discussion presented in this paper supports the Morhenn's hypothesis further through ascribing a direct role to NO in inducing keratinocyte hyperproliferation.

Further support for the proposed role of NO in inducing the psoriatic disease process could be provided by considering the NO's ability in suppressing apoptosis: Traditionally, psoriasis has been viewed as a hyperproliferative disorder of keratinocytes. An important breakthrough in the understanding of the pathomechanism of psoriasis was the finding that keratinocytes derived from psoriatic plaques have a prolonged capacity to resist induction of apoptosis compared with normal-skin-derived keratinocytes.[22] This may contribute to the disease process in which there is marked accumulation of cells in the epidermis accompanied by a thickened stratum corneum. Of note is that the anti-apoptotic Bcl x receptor, a member of the Bcl 2 family, is overexpressed in basal psoriatic keratinocytes.[13]

Apoptotic cell death can result either from developmentally controlled activation of endogenous execution programs or from transduction of death signals triggered by a wide variety of exogenous signals. One major path in the cell suicide program requires the activation of cysteine proteases of the interleukin-1$\beta$-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like family. These proteases play a pivotal role not only in TNF-alpha and APO-1/Fas-triggered apoptosis, but turn out to be of general importance in the apoptotic-signalling cascade.[23]

Though high concentrations of NO (>300 μM) trigger apoptosis through direct DNA damage or inhibition of cellular enzymes by binding to their iron-sulphur moieties, it has been shown that NO, at low concentrations (< 50 μM), is capable of suppression of apoptosis, independent of elevation of cGMP levels and via inhibition of interleukin-1$\beta$-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases by S-nitrosylation of the functionally essential cysteine groups conserved among ICE/CPP-32-like proteases.[23] Furthermore, NO is known to

exert antiapoptotic effect through increasing the expression of protective genes such as heat shock protein and Bcl-2[24]and also through cGMP-dependent inhibition of acid sphingomyelinase which contributes to activation of the initiator capsase-8 and early DNA fragmentation.[25]

Therefore, it could be concluded that NO, having an estimated maximum concentration of 0.01 μM in the psoriatic lesions, may induce the psoriatic disease process not only through induction of keratinocyte hyperproliferation but also via suppression of keratinocyte apoptosis.

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