

Adulterated Effects of Nitric Oxide-Generating Donors

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Impaired cutaneous nitric oxide (NO) production is associated with diminished antioxidative capacity in skin cells, hindered wound healing, unbalanced inflammatory reactions, and disturbed immunological responses. Use of topically applied NO donors might represent an auspicious new therapeutic approach in the field of dermatology. But what is the appropriate NO-generating compound or system? In this issue, Mowbray *et al.* describe a new chemical inert NO donor that *per se* produces little inflammation in the skin.

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NO is synthesized endogenously by NO synthases (NOS), an enzyme family consisting of three members: constitutively expressed neuronal NOS, endothelial NOS (eNOS), and a cytokine-inducible isoform (iNOS). Several lines of evidence indicate that NO is involved in several physiologic and pathophysiologic reactions in skin, e.g., hair growth, proliferation and differentiation of epidermal cells, wound-healing processes, microbicidal activities, antigen presentation, allergic skin manifestations, regulation of innate immune reactions, and inflammatory responses. In addition, NO also affects ultraviolet (UV) radiation-induced processes, such as melanogenesis and erythema and edema formation. Furthermore, NO is an effective inhibitor of lipid peroxidation, and the coordinated action of NO on the expression of cell-protecting genes as well as preservation of membrane function may play an important role in the protection against UVA- or reactive oxygen species-induced apoptotic or necrotic cell death (for review see Suschek *et al.*, 2006).

In human skin NOS-dependent production of NO potentially occurs in all cell types by at least one of the three NO synthases. Because NO is a radical, its sphere of influence is limited to sev-

eral cell diameters. Thus, NO is a local mediator that does not need complex metabolism for clearance; it is simply diluted and then oxidized to nitrite and nitrate over time. Storage pools for NO do exist: *S*-nitrosothiols, *N*-nitroso compounds, and nitrite are among the most important. This is of particular significance because, during challenge of normal human skin with environmentally relevant UVA doses, nitrite and nitroso compounds decompose and form NO, enzyme independently, reaching considerably higher concentrations than are found with maximal iNOS activity in cytokine-activated human keratinocyte cultures *in vitro*. These findings show that human skin biology uses naturally occurring NO donor molecules, which during sunlight exposure may lead to non-enzyme-derived high-output NO formation. Furthermore, in the acidic milieu of human skin, acid-induced decomposition of nitrite represents an additional source of nonenzymatic cutaneous NO production (for review see Suschek *et al.*, 2006). Pharmacologic intervention with nitrite-derived high-output NO after acidification appeared to be a promising treatment for conditions such as infectious diseases of the skin or the vasoconstriction of Raynaud's

disease, in which significant vasodilatation has been demonstrated using this NO donor system (Tucker *et al.*, 1999). However, topical application of acidified nitrite has been found to induce an intense cutaneous inflammatory infiltrate (Ormerod *et al.*, 1999). Together with the finding that iNOS is expressed in many inflammatory diseases, including a number of inflammatory dermatoses, the far-reaching paradigm of NO as a strong pro-inflammatory mediator in human skin has been established.

NO donors have a wide range of potential uses in skin. Benefits of topically applied NO have been demonstrated in Raynaud's syndrome, and other conditions that involve diminished skin blood flow, such as poorly vascularized skin grafts, could also benefit. An exciting prospect is the potential to accelerate wound healing, which is delayed in both iNOS- and eNOS-knockout mice. In addition, poor wound healing in diabetic mice is associated with decreased eNOS protein expression as well as decreased NO synthesis (for review see Weller, 2003). Even a small acceleration in wound healing would be significant, and data from animal studies are highly suggestive that NO-based therapies may be worthy candidates for clinical studies. However, the effects of NO derived from NO donors depend on localization and the kinetics of release. High-output bursts of NO, such as those released by the chemical reduction of nitrite, may be ideal for their microbicidal effects but not necessarily for other purposes.

The ubiquity of NO in mammalian physiology, although promising for many pharmacologic benefits, is at the same time an Achilles heel. The two major practical problems are lack of specificity, which may result in unwanted side effects, and the lack of adequate pharmacokinetically appropriate and localized means of NO delivery. A wide range of NO donors with different modes and rates of NO release are on the market that release NO either spontaneously or after enzymatic conversion. NO donor drugs can be tailor-made to suit a particular disease target. Long-term use of most clinically used NO donors,

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however, is often limited by tolerance and toxicity issues, ensuring the need for novel alternatives (for review see Miller and Megson, 2007).

In the 1960s the American chemist Russell Drago synthesized and characterized several adducts of NO with secondary amines and called them NONOates, now known as diazeniumdiolates. The advantage of this class of NO donors is that they can be tailored to release NO within seconds, minutes, hours, or even days (for review see Keefer, 2005). These polyamine NO adducts have proved extremely popular in the experimental setting because of their predictable NO release. NONOates have been employed successfully in a number of cardiovascular disorders and diseases, especially when slow, prolonged release is desired, and there is great interest in promoting tumor regression by delivering cytotoxic levels of NO to cancer cells via NONOates. Therefore, a series of NONOates has been designed with protected diolate groups that release NO upon specific enzymatic reactions in tumor cells. However, because many polyamines are corrosive, the potential toxicity of the parent compound and by-products of NONOate metabolism remains to be investigated before topical application of these compounds can be considered. *S*-nitrosothiols are another group of NO donors that can be designed to release NO at varying rates. They decompose cleanly with release of NO and formation of the respective disulfide. *S*-nitrosothiols occur naturally in blood plasma (e.g., as *S*-nitrosoglutathione), may play some part in the normal metabolism of NO, and may act as NO storage compounds. Alterations in the structure of *S*-nitrosothiols to modify lipophilicity may allow these compounds to target areas of interest, minimizing their side effects. Polymeric materials containing *S*-nitrosothiols hold a great deal of promise as coatings for stents, extracorporeal circuits, and catheters used in interventional cardiologic procedures, surgery, and renal replacement therapy. In recent years many new hybrid NO donor drugs have been developed. The concept behind them is best described as NO-donating nonsteroidal anti-inflammatory drugs. These compounds retain the actions of their

parent compounds (analgesia, inhibition of inflammation, decreased thrombosis) but have additional beneficial actions attributed to NO, e.g., protecting against gastric damage caused by aspirin. The concept has been extended to a vast array of pharmacologic agents such as anticoagulants, antitumor agents, ion channel inhibitors, angiotensin-converting enzyme inhibitors, and histamine receptor inhibitors (for review see Miller and Megson, 2007). It remains to be seen whether any of these agents can be used to treat skin diseases or disorders. With conventional NO donors, a spontaneous or induced decomposition leads not only to release of the NO radical but also to the formation of more or less bioactive parent compound(s) or additional chemically reactive species (Scatena *et al.*, 2005).

Nitric oxide may be released in skin from bound complexes with hydrated aluminosilicates.

For example, one concern about the clinical use of NONOates is simultaneous release of the secondary amine. Because NO in an oxygen-rich environment can readily form nitrosating species (probably N_2O_3), formation of carcinogenic secondary nitrosamines may occur. Additionally, polyamines *per se* might exhibit significant biologic effects that support or counteract the beneficial effects of NO. With respect to *S*-nitrosothiols, if the parent compound is a naturally occurring molecule, these components are without hazard. Unfortunately, the most simple one, *S*-nitrosocysteine, is quite unstable. Therefore, much experimental research has been carried out using *S*-nitroso-*N*-acetylpenicillamine; however, the parent compound *N*-acetylpenicillamine is an antibiotic. Even physiologically occurring thiols are not biologically inert. Glutathione disulfide, obtained after decomposition of *S*-nitrosoglutathione, may be reduced enzymatically to glutathione, which is an important redox-active compound that may support the protective properties of NO. On the other hand, sulfur-

centered glutathyl radicals have been shown to promote the oxidation of phospholipids in living cells and to cause cell death. Photo-induced decomposition of nitrite results in NO generation, but also in the generation of hydroxyl and nitrogen dioxide radicals, both of which are highly toxic compounds (for review see Suschek *et al.*, 2006).

Among the topically applied NO donors are polyethyleneimine cellulose NONOates (Shabani *et al.*, 1996) and *S*-nitrosothiols such as *S*-nitrosated thiomonosaccharides (Khan *et al.*, 2003), low-molecular-weight *S*-nitrosothiols embedded in a hydrogel (Seabra *et al.*, 2004), and poly-*S*-nitrosated polyesters (Seabra *et al.*, 2005). In animal models, NO gas has been used to treat wounds (Shekhter *et al.*, 2005) and skin infections (Ghaffari *et al.*, 2007). However, NO gas is notoriously difficult to handle with respect to complete exclusion of oxygen to prevent oxidation to nitrogen dioxide. In this issue of *Journal of Investigative Dermatology* Mowbray *et al.* (2008) report that application of pure NO in a controlled manner *per se* produces little inflammation in human skin. This is in contrast to the inflammatory effects resulting from use of a cream containing an acidic mixture of ascorbic acid and nitrite. In a novel approach to storing and delivering NO to the skin, the authors used zeolites, hydrated aluminosilicates with an open structure ("molecular sieves") that can accommodate a wide variety of cations. Exposure of these solids to NO gas results in NO binding to the metal ions within the pores, facilitating highly efficient packing of NO. NO-zeolites of this type are very stable in the anhydrous state, but NO is displaced when they contact water. The beauty of zeolites is that chemically they are completely inert, they constitute very-high-capacity stores for NO, and their rate of release can be modulated by altering the chemical zeolite composition (Wheatley *et al.*, 2006). The infinite flexibility of NO release allows the development of a wide range of different NO donor materials for purposes ranging from fast-acting antimicrobial coatings for urinary catheters and wound dressings to durable, slow-acting antithrombotic coatings for stents, bypass tubing, cannulae, and catheters.

Although still in an early stage, this approach represents a novel and simple way to optimize the benefits of topical NO as a local biologic mediator.

Although NO-releasing zeolites may not be the last answer, it is to the credit of Mowbray *et al.* that they have designed a system of applying pure NO in a controlled and predictable manner without causing an inflammatory reaction in human skin. Wound healing is a particularly interesting application, given the multiple benefits that NO might have, depending on its concentration and the kinetics of delivery. Central to the success of NO donor therapy is generation of the correct amount of NO in the right place for the right length of time. Solving these problems will undoubtedly lead to new NO donor therapies for skin diseases and disorders.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Neuroimmune Communication in Skin: Far from Peripheral

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The precise nature of the link between stress and exacerbation of skin inflammation has puzzled researchers and clinicians alike. The traditional explanation is that immune balance is altered by activation of two stress axes: activation of the hypothalamic–pituitary–adrenal (HPA) axis raises cortisol levels, and activation of the sympathetic nervous systems raises adrenaline levels (Figure 1). In this issue, Pavlovic *et al.* (2008) provide evidence for a third stress axis and report that stress travels to the skin through peripheral neuropeptidergic nerve fibers and exacerbates the neurogenic inflammatory aspect of cutaneous dermatitis. Clarification of the role of this alternative stress axis may enable the design of novel therapeutic strategies.

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Pathologies that involve chronic relapse and significant discomfort, such as atopic dermatitis, are regarded as psychosomatic dermatoses (Wright *et al.*, 2005). In the early days of psychoanalysis, the skin was regarded as one vehicle by which the body and soul express the feelings and well-being of its bearer. Itchy and inflamed skin was interpreted as a cry for attention and help and a sign of severe deprivation of tenderness and care.

In the past, the close interaction between neuropeptidergic nerve fibers and immune cells in the skin was widely understood as playing a role in the cutaneous stress response. Altered nerve-fiber counts in atopic dermatitis, psoriasis, nummular eczema, and many

other—mostly chronic and inflammatory—skin pathologies were viewed as evidence of the possible influence of stress on the course of inflammation.

In parallel, stress research provided evidence of a close interaction between nerve fibers and, for example, mast cells under stress (ranging from physical to psychological) (Theoharides and Cochrane, 2004). The idea that mast cells are activated under conditions of stress in exacerbated inflammatory skin disease became accepted almost to the point of textbook knowledge, even though experimental evidence has still not been provided.

Initial insights into the pathogenesis of stress-induced exacerbation of

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