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New Aspects of Nitrite Homeostasis in Human Skin

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Enzymatic as well as nonenzymatic mechanisms generate nitric oxide (NO) from nitrite in the blood, stomach, saliva, and urine, as well as in the skin. In human skin, the inorganic anions nitrate and nitrite are not inert end products of endogenous nitric oxide metabolism, as long believed, but can be recycled by environmental stimuli such as UVA radiation to form NO; thus, they represent an important alternative nonenzymatic physiological source of NO.

Journal of Investigative Dermatology (2009), **129**, 820–822. doi:10.1038/jid.2009.11

Several lines of evidence indicate that nitric oxide (NO) is involved in various physiological as well as pathophysiological reactions in the skin, including hair growth, proliferation and differentiation of epidermal cells, control of wound healing, microbicidal activity, antigen presentation, allergic skin manifestations, regulation of innate immune reactions, and inflammatory responses. In addition, UV-induced processes such as erythema and edema formation, as well as melanogenesis, are affected by NO (for review, see Weller, 2003). 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 protection against either UVA- or reactive oxygen species-induced apoptotic or necrotic cell death (for review, see Suschek *et al.*, 2006). NO is endogenously synthesized by NO synthases (NOSs), an enzyme family consisting of three members, constitutively expressed neuronal NOS, endothelial NOS, and a cytokine-inducible isoform.

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 extends only to several cell diameters. Therefore, NO truly 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. However, storage pools for NO do exist, with S-nitrosothiols, N-nitroso compounds, nitrite, and nitrate being the most important. The inorganic anion nitrite (NO_2^-) was previously thought to be an inert end product of endogenous NO metabolism. However, recent studies demonstrated that this supposedly inert anion can be recycled *in vivo* to form NO, representing an important alternative source of NO to the classic L-arginine–NO synthase pathway (Suschek *et al.*, 2006).

In blood, nitrite contributes to blood flow regulation by reaction with deoxygenated hemoglobin and tissue heme proteins to form NO, and plasma nitrite levels decrease progressively with increasing cardiovascular risk, such as hyperlipidemia, arterial hypertension, and smoking. Nonenzymatic NO generation from nitrite has been reported at several other sites in humans. In the stomach, NO is formed from nitrite at acidic pH, which requires continuous delivery of saliva-containing nitrite. NO increases mucous barrier thickness and gastric blood flow and shortens the time required to kill ingested pathogens within the normal residence time of food in the stomach. A similar mechanism of NO generation from acidified salivary nitrite has been detected in the oral cavity, where NO is suggested to play a role in host defense. Acidification of urine, such as occurs following intake of vitamin C, results in NO generation from urinary nitrite and acts as a bacteriostatic, with respective consequences in urinary bladder pathophysiology (for review, see Lundberg *et al.*, 2008).

Application of near-physiological nitrite concentrations results in NO-dependent cytoprotection from cardiac and liver ischemia–reperfusion injury in mice. In addition, a 2-week infusion of nitrite prevented delayed cerebral vasospasm in a primate model of subarachnoid hemorrhage, suggesting that nitrite serves as a backup source of NO, buffering tissue temporarily against severe hypoxia and providing protection via compensatory vasodilation.

In the context of skin physiology, nitrite action is of particular importance. During challenge of normal human skin with environmentally relevant UVA doses, nitrite and nitroso compounds decompose and enzyme-independently form NO at manifold higher concentrations than are found with maximal inducible NOS activity in cytokine-activated human keratinocyte cultures *in vitro*. These findings indicate that human skin uses naturally occurring NO donor molecules, which during sunlight exposure will 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 non-enzymatic cutaneous NO production (for review, see Suschek *et al.*, 2006). Pharmacological 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, where a significant vasodilatation has been demonstrated using this NO donor system (Tucker *et al.*, 1999).

In the past 20 years, an ongoing discussion in the NO research field has included attempts to typify the physiological or pathophysiological character of NO: is it good, bad, or ugly? The cellular response to increases in intracellular

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NO concentration seems to depend largely on the redox potential of the cell, which is itself influenced by the resting levels of NO. In human skin cells and tissues, even supraphysiological high NO concentrations were shown to protect cells from oxidative stress and UVA-induced apoptosis. In other cell systems, NO promoted apoptosis even at "physiological" concentrations, earning it the epithet "the Janus-faced molecule."

But what is the character of nitrite in human skin as a potential NO donor during UVA exposure? In rat endothelial cells, nitrite-derived NO formation represented an effective protective principle against UVA-induced cell injuries, even at concentrations of 10 mM nitrite (Suschek *et al*., 2003). Further, physiological intracellular nitrite concentrations significantly reduce the susceptibility of human skin fibroblasts to UVA-induced cell death. Thus, fibroblasts depleted of intracellular nitrite die at significantly lower UVA doses than fibroblast cultures supplemented with skin-physiological nitrite concentrations of 10 μM (Opländer *et al*., 2008). In this setting, skin-derived nitrite seems to be the "good guy," and it seems feasible that in special circumstances nitrite and other sources of enzyme-independent NO formation may play a pivotal role in human skin physiology, as is postulated for NO itself. On the other hand, with human skin fibroblasts, experiments identical to those performed with rat endothelial cells mentioned above reveal that nitrite at supraphysiological concentrations of 100 μM readily augments UVA-induced cell death because of the simultaneous generation of highly toxic $\bullet NO_2$ radicals, a side product of UVA-induced nitrite decomposition (Opländer *et al*., 2007). Then, is nitrite the "bad guy"? Although cutaneous NO formation via photodecomposition of nitrite represents an effective antioxidative and protecting role for NO, the simultaneous production of $\bullet NO_2$ represents a strong toxic insult. Thus, depending on the individual antioxidative equipment of a cell type, during UVA exposure nitrite might protect against UVA-induced oxidative stress or enhance the injurious effects of UVA. Nitrite, as NO, has a redox-dependent Janus-faced character.

> NO has a redox-dependent "Janus faced" character.

Because of nitrite's Janus characteristics, it is important to know more about the physiological concentrations of cutaneous NO-related products, as well as the possible regulatory mechanisms of cutaneous nitrite homeostasis. It has been postulated that the amount of skin-derived NO derivates solely reflects the NO amount produced by skin cells. It was assumed that some of the NO molecules formed in skin remain at or close to the point of generation in the chemical form of S- or N-nitroso compounds or as the oxidation products nitrite or nitrate. Concentrations of up to 10 μM nitrite were detected in human skin specimens, representing a more than 25 times higher concentration of these compounds than that reported in the plasma of healthy volunteers (Paunel *et al*., 2005). In this issue, Mowbray *et al*. present *in vivo* data that confirm and quantify the presence of NO-related products in human epidermis, superficial vascular dermis, and skin surface sweat. These species have been shown to exhibit an ability to release NO during UVA exposure, findings that are of great significance in the context of the skin as the largest "organ" of the human body (10–20 dm^3), which thus offers a considerable storehouse. A further surprising and innovative finding is the discovery of a relationship between the concentration of NO-related products in plasma and those in the superficial vascular dermis and sweat.

Mowbray *et al*. (2009) further describe a strong interindividual variation in the concentration of NO-related products in saliva, plasma, sweat, epidermis, and superficial vascular dermis that was similar in each of the biological samples. Gladwin *et al*. (for review, see Gladwin *et al*., 2005) suggested that the majority of nitrite in tissues originates from the exogenous intake of nitrite and nitrate and not from endogenous sources, thus resulting in great variation in tissue nitrite levels with nitrate and nitrite intake. In contrast, plasma levels of nitrite vary only slightly, suggesting the existence of regulatory pathways in blood (Bryan *et al*., 2005).

The main sources of nitrate and nitrite in our diet are green vegetables (such as lettuce and spinach), root vegetables, cured meat, and water. In various European countries and the United States, estimated dietary intakes of nitrate and nitrite range from 31 to 185 mg/day and from 0.7 to 8.7 mg/day, respectively. Upon rigorous exclusion of dietary nitrate, urinary excretion in human volunteers is about 1 mmol nitrate per day (i.e., an amount similar to that provided by food). This suggests that the amount of nitrate synthesized endogenously by NO synthases is comparable to the amount of nitrate ingested.

Because NO-related products in plasma can be influenced by dietary intake of nitrate or nitrite, Mowbray *et al*. (2009) postulate that dietary NO derivates may offer a source for manipulation of cutaneous NO-related products and that an individual's dietary consumption of green leafy vegetables may, in part, influence his cutaneous response to UV radiation. It is well known that vegetarians are at a reduced risk of developing hypertension and other cardiovascular diseases, and it has been speculated that the high nitrate/nitrite content of many vegetables may contribute to these cardioprotective effects. The findings described by Mowbray *et al*. (2009) strongly suggest an analogous role for nitrite/nitrate-containing nutrients in the protection against the injurious inflammatory or carcinogenic properties of pro-oxidative environmental stimuli such as UVA radiation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Bullous Pemphigoid: A Prototypical Antibody-Mediated Organ-Specific Autoimmune Disease

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Bullous pemphigoid (BP) is a prototypical organ-specific autoimmune disease. Autoantibodies unfold their blister-inducing potential by triggering an Fcγ-dependent inflammatory reaction. The study by Iwata *et al.* in this issue provides the first direct evidence that IgG autoantibodies from BP patients may also weaken cell–matrix adhesion by depleting BP180/type XVII collagen from cultured keratinocytes. These novel findings shed new light on additional mechanisms of blister formation in pemphigoid diseases and open the way for further informative studies.

Journal of Investigative Dermatology (2009), **129**, 822–824. doi:10.1038/jid.2009.12

Bullous pemphigoid (BP) is a subepidermal blistering disease that typically affects the elderly and is associated with an autoimmune response against hemidesmosomal proteins (Liu and Diaz, 2001; Mihai and Sitaru, 2007; Olasz and Yancey, 2008). Extensive clinical and experimental evidence strongly suggests that autoantibodies cause the pathology in this disease (Sitaru and Zillikens, 2005; Leighty *et al.*, 2007). Circulating autoantibodies in BP patients exhibit a heterogeneous specificity to several hemidesmosomal components, including BP230, an intracellular constituent of the hemidesmosomal plaque, and the transmembrane protein BP180/type XVII collagen (Sitaru and Zillikens, 2005; Leighty *et al*, 2007). Mutations in COL17A1 in patients with generalized atrophic benign epidermolysis bullosa and in knockout mice result in low or absent expression of BP180/type XVII collagen and subepidermal blistering (McGrath *et al.*, 1995; Nishie *et al.*, 2007). These findings suggest that a decrease in the expression of this hemidesmosomal antigen may weaken cell–matrix adhesion in the skin and eventually result in dermal–epidermal separation.

Autoantibodies to the transmembrane antigen BP180, but not to the intracellularly located BP230, were hypothesized to be pathogenically relevant (Liu and Diaz, 2001; Sitaru and Zillikens, 2005). Indeed, experimental evidence generally supports the pathogenic role of autoantibodies to BP180 for blister formation. IgG autoantibodies, affinity purified against recombinant BP180 from patients with BP and pemphigoid gestationis, induce dermal–epidermal separation in cryosections of human skin when co-incubated with leukocytes from healthy donors (Sitaru and Zillikens, 2005). In 1993, Liu *et al.* first provided evidence for a pathogenic role of autoantibodies to type XVII collagen/BP180 *in vivo* (Liu *et al.*, 1993). The authors demonstrated that rabbit antibodies generated against murine BP180 induce subepidermal blisters when passively transferred into neonatal mice. More recently, further animal models reproducing blister formation in BP have been developed using mice that express the human form of the BP180 antigen injected with BP patient autoantibodies. Studies performed mainly with the experimental model developed by Liu *et al.* (1993) revealed that subepidermal blistering triggered by rabbit IgG specific to murine BP180 depends on complement activation, mast cell degranulation, macrophage activation, and neutrophilic infiltration. Reactive oxygen species and proteases, such as gelatinase B/MMP-9 and elastase, are critically involved in blister formation *in vivo* and *in vitro* (Liu and Diaz, 2001; Liu, 2004; Sitaru and Zillikens, 2005; Leighty *et al.*, 2007). These findings partially match the pathology observed in BP patients and support the prevailing

> IgG autoantibodies deplete BP180 from keratinocytes.

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