

The Basis of Topical Superoxide Dismutase Antipruritic Activity

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SUMMARY In humans, as in all mammals and most chordates, three forms of superoxide dismutase (SOD) are present: SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular. SOD is used in cosmetic products to reduce free radical damage to the skin, for example, to reduce fibrosis following radiation for breast cancer. Pruritus is one of the most common symptoms of skin diseases, but can also be a major symptom of systemic diseases (e.g., malignancy, infection or metabolic disorders). There are various antihistaminics used as antipruritogenic substances. In the genesis of pruritus there are many pruritogens involved, not only histamine and leukotrienes such as acetylcholine, cytokines, kallikreins, proteases, kinins, opioids, etc., which are described. On many occasions, we observed that topical SOD seemed to possess strong antipruritic activity, even in anti-histamine-resistant pruritus. We analyzed literature data on the effect of SOD as an anti-pruritogen on NK-1 receptors and proinflammatory cytokines, its regulatory role in calcitonin gene-related peptide production and expression, down-regulation of TNF- α and numerous cytokines, and suppression of nitric oxide production.

KEY WORDS: pruritus, itch, topical superoxide dismutase, opioids

INTRODUCTION

Despite its common use, the word pruritus is not easy to define. A simple description is that pruritus is an unpleasant cutaneous sensation that provokes a desire to scratch (1). However, despite a century of research and investigations on pruritus (2), there is no generally accepted therapy for the treatment of itch, and many mysteries and controversies still haunt this niche in the life sciences (3).

This simple attempt to define pruritus is certainly not perfect, as Savin in 1998 noticed that "the word unpleasant means different things to different people", and patients suffering from itch do not always desire to scratch (4). Pruritus can be a physiological sensation if the consecutive scratching removes the potential agent, or pathological if associated with skin and/or internal diseases and mental disorders, or caused by some food

or drugs (1). We will use the terms pruritus and itch as synonyms, but some authors use the term itch when skin lesions are present and pruritus if there are no primary skin alterations (5). Pruritus can be experienced only in the skin because of the unique neural mechanisms it involves, but this is in fact an extracutaneous event, a pure product of the central nervous system (CNS) activities.

Pruritus has to be distinguished from pain, although there is close relation between itch and pain (3). In the same manner, the sensation of itch and nociception (touch) are distinct entities. Pruritus by itself is a symptom, but not a disease. It can affect equally patients of all ages and both sexes. Its intensity can be mild, moderate, severe and even distressing with sleep disturbances, loss of weight, discomfort, increased irritability, problems in daily activities and even stress (1). It can be acute or chronic, sometimes long lasting, and may affect any part of the body. Pruritus is not limited to humans, but can affect nearly all mammals. It may present a diagnostic challenge to the clinician, be it a dermatologist, family doctor, internist, pediatrician or psychiatrist. Although it may seem a minor symptom compared to the severity of some diseases, pruritus must be taken in consideration by the physician because most often it can affect very seriously the patient quality of life.

THE CAUSATIVE FACTORS OF PRURITUS

Pruritus may be provoked by both exogenous agents and endogenous causes or stimuli. It ap-

pears that the chorus of itch-inducing agents contains many more protagonists than the usual and historical suspect, histamine (3). The excellent review by Paus *et al.* (3) gives a summary of major pruritogens, with the receptors interacting in the genesis of pruritus (Table 1). As it can be observed, apart from histamine, the list includes several pruritogens, not yet commonly viewed as pruritic agents known for a long time, such as proteases, leukotrienes or cytokines. It seems pretty sure that in the future, many more biological agents will be added to the list. The role of histamine, in contrast, was long overestimated (3); small doses of histamine can fail to produce itch, while being sufficient to cause edema and erythema upon intracutaneous injection, and no sedative antagonists of the histamine receptors H1 and/or H2 have often been proven to be of low or no efficacy as antipruritic drugs (3). Besides the army of chemicals and various substances susceptible of exogenously induced pruritus, a number of pathologic processes can lead to itch, e.g., inflammation, hypersensitivity, degenerative changes, malignant tumors and even mental abnormalities (1).

THE PATHOGENESIS OF PRURITUS

The neuroanatomic basis of pruritus is relatively well known. Itch originates in free nerve endings, near the dermoepidermal junction, and is conducted centripetally by afferent nerves entering the spinal cord *via* dorsal roots. The sensitive nerves for pruritus are small, non-myelinated C fibers with a slow conduction rate. The cell bodies

Table 1. Main pruritogens modified from Paus *et al.* (3)

Pruritogen	Receptors
Acetylcholine	Nicotinergic (nAChR) and muscarinergic (mAChR) receptors
CGRP	CGRP receptors
CRH and POMC	CRH-R1 and CRH-R2
Cytokines	Cytokine receptors (IL-1, IL-31,...)
Endocannabinoids	CBs (CB1, CB2)
ETs	ET receptors
Endovanilloids	Activation of TRPV1 and sensitization of TRPV1 <i>via</i> activation of specific receptors
Histamine	Histamine receptors (H1R-H4R)
Kallikreins, Proteases	Partly by PARs
Kinins	Bradykinin receptors (B1R, B2R)
Leukotriene B4	Leukotriene receptors
NKA and SP	Tachykinin receptors
NKA, BDNF, NTs	Specific receptors: TrkA (NGF), TrkB, (NT-4, BDNF), TrkC (NT-3)
Opioids	Opioid receptors

CB – cannabinoid receptor; CRGP – calcitonin gene-related peptide; CRH – corticotropin-releasing hormone; POMC – pro-opiomelanocortin; ET – endothelin; NKA – neurokinin A; SP – substance P; PAR – proteinase-activated receptors; BDNF – brain-derived neurotrophic factor; NT – neurotrophin; TRPV-1 – transient receptor potential vanilloid-type 1; Trk – tyrosine kinases: A, B, C; NGF – nerve growth factor; NT-3 – neurotrophin-3; NT-4 – neurotrophin-4

of these nociceptive primary neurons are located in the dorsal root ganglion. After entering the spinal cord, the primary neurons synapse secondary neurons whose axons cross to the opposite side, and then by the tractus spinothalamicus reach the laminar nuclei of the thalamus. Finally, these nuclei relay to the cerebral cortex, i.e. the sensory area in post central gyrus. Here we note the location, the nature, the intensity and other qualities of this sensation (1).

For a long time, itch was considered as a weak pain, but recently the existence of specific C fibers for pruritus has been suggested (5). Interestingly, recent works suggest that the epidermis itself, especially the keratinocytes that form the bulk of the epidermis, constitute the itch receptor (6). Keratinocytes express a range of neuropeptide mediators and receptors that appear to be involved in pruritus, including opioids, nerve growth factor (NGF), substance P (SP) and receptors including vanilloid receptors and protease activated receptors type 2 (PAR-2). Thus, the epidermis and its associated ramifications of fine intraepidermal C-neuron filaments can be looked upon as the "itch receptor" (7). Crosstalk between C neuron terminals and the spatially closely related dermal mast cells is increasingly recognized as being important in the pathophysiology of itch (7).

THE MEDIATORS OF PRURITUS

Pruritus is caused by the release of mediators acting peripherally on receptors, cells or nerves. Some of these substances can act directly on the free nerve ending, whereas others act indirectly through mastocytes or other cells, in particular keratinocytes. The traditional histamine, an imidazolethylamine, was the first and most important recognized pruritogenic substance, but it does not account for all types of pruritus. It is released from the metachromatic granules of mast cells and then acts on the receptors eliciting itch (8,9). Applied intraepidermally or at the basal membrane level, it causes itch, while released in the dermis it causes pain and edema. It acts on the neurons by increasing the levels of CAMP. Recently, a new class of histamine receptor, H4, operating as a transducer of itch, has been identified in mice (10). Histamine does not seem to be involved in pruritus accompanying psoriasis, insofar as there was no correlation observed between pruritus intensity and histamine plasma levels in psoriasis or in histamine plasma levels between pruritic and non-pruritic patients with psoriasis (11). Most of psoriasis patients claim that antihistamines are not effective

in reducing pruritus and not even in preventing it (12,13).

Serotonin (5-hydroxytryptamine-5-HT), injected intradermally can also cause pain and pruritus (13). PGE2 does not elicit itch itself, but lowers the threshold and potentiates the itch provoked by histamine (14). Mast cells can produce two proteases, chymase and tryptase. Tryptase has recently been shown to activate PAR-2 expressed on afferent C neuron terminals (15), stimulating the sensation of itch and also triggering the release of SP. This is in accordance with the fact that substances such as papain, trypsin, chymotrypsin or kallikrein are known for a long time as causing pruritus when injected in the skin (16). SP is a pro-inflammatory neuropeptide consisting of eleven amino acids, produced in the dorsal ganglia and then transported to the periphery by nociceptive nerves A and C (17). SP-reactive fibers are localized close to mast cells (18) and hence release of SP from sensory afferents can stimulate mast cell secretion *in vivo* (19) SP degranulates mast cells and therefore can release histamine from them, provoking itch (20). Hence, it is commonly used to induce experimental scratching (21,22). When compared with psoriatic patients free from pruritus, lesional skin from patients with pruritus showed an increase in SP-containing nerve fibers in perivascular areas (23), and keratinocytes in the psoriatic plaques of patients with pruritus showed consistently increased expression of SP-receptor compared to non-pruritic patients (24). On the other hand, it could be observed that serum levels of SP correlated with the clinical score and quality of life score in patients with atopic dermatitis (25). The involvement of SP in the stress response of the skin has been also acknowledged (26) and the SP action may be mediated at least partly by cutaneous NK1 receptors (27).

In the skin, SP can also cause erythema, edema, and neurogenic inflammation releasing IL-1, prostaglandin and lysosomal enzymes (28-30). The proinflammatory activity of SP was also demonstrated in neurogenic bladder, in which its ability to stimulate reactive oxygen species (ROS) generation was enhanced (31). In human neutrophils (32), SP primes two distinct pathways with respect to the induction of ROS: the production of superoxide anion and hydrogen peroxide by the calmodulin-dependent NADPH oxidase, and the generation of nitric oxide (NO) by constitutive NO synthase. SP was also shown to induce superoxide anion production in monocytes from patients with rheumatoid arthritis (33), but also with intersti-

tial long disease (34), the latter in a dose-dependent manner.

Induction of NO production by SP was demonstrated in the neutrophils and cardiac endothelium of Mg-deficient rats (35).

Intradermal SP injection was shown to increase cutaneous NO (36,37), enhancing SP-induced itch-associated responses. This SP action may be mediated at least partly by cutaneous NK1 receptors, which could be involved in mediating scratching (38).

On the other hand, SP was found to increase tumor necrosis factor alpha (TNF- α) secretion from human peripheral blood mononuclear cells (39), to cause significant increase in TNF- α production by HSV-infected mouse peritoneal macrophages (40), in the whole blood cells of rheumatoid arthritis and osteoarthritis patients (41) and to induce TNF- α mRNA expression in human monocytes of patients with rheumatoid arthritis (33). In cases of whole blood cells from patients with rheumatoid arthritis (41) and human peripheral blood mononuclear cells (39), SP was also responsible for a significant increase of interleukin-6 (IL-6) production. Interleukin-1 (IL-1) production was also found to be increased in the same studies (39-41). Besides, in murine keratinocyte PAM 212, SP augmented the expression of IL-1 α mRNA and the secretion of bioactive IL-1 in a dose-dependent manner (42).

Another neuropeptide deserving attention in the pathogenesis of pruritus is probably calcitonin gene-related peptide (CGRP). A significantly elevated plasma level of CGRP was clearly documented in pruritic psoriasis compared with healthy individuals (43). Such a difference was not observed when non-pruritic subjects were compared with the control group. Significantly higher CGRP concentration was also observed in atopic dermatitis patients suffering from severe pruritus (44). Staining for CGRP showed a large increase of immunoreactive nerves in lesional skin of nodular prurigo patients (45). Skin-scratching behavior is a common response observed in patients with pruritus, and was shown to dramatically induce the sprouting of cutaneous nerve fibers; at the same time, nerve fibers containing SP and CGRP increased significantly (46). The bad thing is that each neuropeptide (SP and CGRP), alone or in coordination with the other, can cause significant increase in IL-1 β and TNF- α production, as demonstrated in HSV-infected mouse peritoneal macrophages (40).

Neurotrophins, the key prototype of which is the nerve growth factor (NGF), are also key molecular players in the pathogenesis of itch (6). Basal epidermal keratinocytes in humans express NGF (47), but also fibroblasts *in vitro*, stimulating fibroblast migration (48).

Normal human keratinocytes synthesize and secrete biologically active NGF (47), while NGF is released in increasing amounts by proliferating keratinocytes, whereas secretion ends in more differentiated cells (49). Hence, NGF is copiously produced by the skin epithelium in order to direct and control sensory skin innervation (50). The involvement of NGF in the stress response has recently been acknowledged (51), in which it is now recognized as an important parameter (19). NGF directly stimulates activation of mast cells via functional neurotrophin receptors, degranulation of the same and release of cytokines, thereby promoting and/or aggravating neurogenic inflammation (52,53). During inflammation NGF stimulates tissue nociceptors and enhances inflammatory pain (54). On the other hand, NGF can promote outgrowth of SP-positive nerve fibers, for example towards mast cells, thereby building up a network that allows for increased neurogenic inflammatory responses upon stress (55). NGF regulates the nociceptive properties of a subset of small diameter sensory neurons by increasing the expression of the heat-sensing transient receptor potential (TRP) channel, TRPV1 (56). Treatment of rat dorsal root ganglion neurons with NGF increased TRPV1 protein expression but not mRNA, and this increase was mimicked by H₂O₂ and attenuated by catalase (56). NGF is thought to be involved in the pathogenesis of prototypic pruritic dermatoses such as prurigo nodularis and atopic dermatitis (57,58). Therapeutic administration of NGF has been found to be pruritogenic (59). NGF expression was shown to be increased on nerve fibers, Schwann cells, mast cells, eosinophils and keratinocytes in the skin affected by allergic contact dermatitis, prurigo and atopic dermatitis, and in the serum of patients with atopic dermatitis (60,61). Expression of NGF in affected skin is significantly increased in conventional NC/Nga Tnd mice with mild to severe atopic dermatitis compared to NGF contents in mice with no dermatitis, suggesting the possible involvement of NGF not only in inflammatory process but also in extension of sensory nerve fibers. NGF produced from proliferating keratinocytes and fibroblasts in atopic skin may invite sensory nerves closely to the epidermis, and may cause itch indirectly (58). Furthermore, NGF

may contribute to constitution of skin hypersensitivity. As regards psoriasis, when compared with psoriatic patients free from pruritus, lesional skin from patients with pruritus showed strong immunoreactivity for NGF throughout the epidermis and an increased NGF content in lesional skin, as well as an increase in the expression of high-affinity receptors for NGF (TrkA) in basal keratinocytes and in dermal nerves (23). NGF can influence many pathologic processes such as proliferation of keratinocytes, angiogenesis, T-cell activation, expression of adhesion molecules, increase of cutaneous innervation and up-regulation of neuropeptides, all known to take place in psoriasis (62). Moreover, preliminary recent data from the murine system suggest that, *vice versa*, potent proinflammatory cytokines like TNF- α , IL-1 and interferon- γ (IFN- γ) can up-regulate the cutaneous expression of NGF, and may thus contribute to the vicious cycle of proinflammatory events maintaining and promoting inflammatory skin diseases, besides pruritus (63). In the same way, NGF expression could be under the influence of ROS, as it was demonstrated that a single exposure to peroxynitrite specifically induced NGF expression and secretion in astrocytes (64). Cytokines are also key molecular players in the occurrence and maintenance of pruritus. It was demonstrated (65) that TNF- α was able to induce SP in sympathetic ganglia through the sequential induction of IL-1. In the same way, HIV patients experiencing pruritus were found to have significantly higher TNF- α levels in their epidermis than pruritus-free HIV patients (66). A high significant elevation of serum TNF- α was also observed in scabies patients compared with controls (67). Infliximab, a human-murine monoclonal anti-TNF- α antibody, used in psoriasis, yields good responses in patients with associated pruritus (68,69). Interestingly, there are three recent publications detailing the ability for suppressing scratching behaviors in mice of three vegetal compounds: glucomanan (70) schizandrin (71) and magnolol/honokiol (72). All three were suppressing concomitantly the overproduction of TNF- α , but also of IL-4 and IL-10.

Interleukins constitute another interesting family, some members of which seem to be deeply involved in the mechanism of pruritus. They interact in the secretion of various neuropeptides or neurotrophins directly or indirectly responsible for pruritus. IL-1 stimulates the production of PGE2 fibroblasts through Cox-2 expression (73,74). IL-1 also up-regulates the expression of NGF (54). As regards the interactions between IL-1 and SP,

there is a bulk of literature available. The stimulation of SP expression by IL-1 β was demonstrated in rat brain endothelial cells (75), excitatory motor neurons (76), primary cultured rat dorsal root ganglion (77), tracheal neurons (78), myenteric nerves of rat intestine (79), and elevated levels of SPmRNA were found after IL-1 treatment of explanted ganglia (80). Intrathecal injection of IL-1 α in mice induced behaviors involving scratching and biting (81). These IL-1 induced behaviors were similar to the nociceptive responses induced in mice by intrathecal injection of SP. IL-1 might play a role by acting as a factor augmenting pruritus transmission by either directly or indirectly releasing SP.

All experiments in dorsal or cervical root ganglia conclude on an increase in SP synthesis (77,82,83) by IL-1, by enhancing the expression of preprotachykinin (PPT) mRNA encoding for SP and other tachykinins. In human astrogloma cells and primary rat astrocytes, IL-1 β up-regulates the expression of neurokinin-1 receptor (NK-1R), the primary receptor for SP in both cell types, at both mRNA and protein levels (84). IL-1 β induces SP release from primary afferent neurons through the COX-2 system (85), as it does for PGE 2 (73,74). A recent paper (86) discloses that human mesenchymal stem cells-derived neuronal cells express the neurotransmitter gene Tac1, but do not synthesize the gene's encoded peptide, the neurotransmitter SP, unless stimulated with the inflammatory mediator, enhancing the key role of the latter in pruritus. In a similar way, toxin B of *Clostridium difficile* increases SP in human VIP submucosal neurons in part *via* an IL-1 β dependent pathway (87). Interestingly, it seems to be a *vice versa* mechanism, as the release of IL-1 α and TNF- α was evidenced upon the addition of SP onto the skin fragments in culture (88). Interleukin-2 (IL-2) given intradermally or i.v. causes itch, even in healthy subjects (89). The effect of IL-2 was investigated on C-fiber nociceptors on rat saphenous nerves (90). There was strong activation of the same by intradermal injection of IL-2, which was dose dependent (91). It was obvious evidence that IL-2 was a potent activator of a discrete population of cutaneous C-polymodal nociceptors, which are chemosensitive to endogenous inflammatory mediators.

In a patient developing intractable pruritus 3 years after beginning hemodialysis, a high increase of serum IL-2 levels was also observed (92). As regards psoriasis, there was an increased number of IL-2 immunoreactive cells in pruritus vs. non pruritic lesions of psoriasis (23). As IL-2 has been shown to induce itch, it has also been shown

that there is a nocturnal increase in the secretion of IL-2 in healthy volunteers, possibly making more susceptible individuals prone to itch (93). IL-6 is another player in the mechanism of pruritus. Serum IL-6 levels were shown to be significantly higher in hemodialysis patients with uremic pruritus than in hemodialysis patients without pruritus (94). On the other hand, a recent paper shows that there could be an important relationship between scratching, sleep quality and IL-6 levels in atopic dermatitis patients (95). Recently, a novel cytokine, interleukin-31 (IL-31), has been discovered that may have a major role in the pruritus process. It was shown (96) that transgenic mice overexpressing IL-31 developed severe pruritus along with alopecia skin lesions. The expression of IL-31 mRNA in the skin of NC/Nga mice with scratching behavior was found to be significantly higher than in NC/Nga mice without scratching behavior (97), IL-31 being responsible for this itch-associated scratching behavior (98). IL-31 was also significantly overexpressed in pruritic atopic compared with non-pruritic psoriatic skin inflammation (99). Highest IL-31 levels were detected in prurigo nodularis, one of the most pruritic forms of chronic skin inflammation.

It is strongly supposed that IL-31 expression is associated with CLA (+) T cells and might contribute to the development of atopic dermatitis-induced skin inflammation and pruritus (100). Other recent studies (101,102) lend strong support to an important role of IL-31 in the pathogenesis of non-atopic eczema, suggesting that altered regulation of IL-31 gene expression is the disease-causing factor. Besides an important role in the etiology of pruritus, IL-31 was found to be responsible for inducing cytokine and chemokine production from human bronchial epithelial cells (EGF, VEGF, IL-6 and IL-8) and plays the role of a mediator for inflammation (103,104).

Vessels and adhesion molecules appear to play a role in the occurrence and development of pruritus, at least in certain dermatologic disorders. E-selectin levels showed a statistically significant correlation with the SCORAD score in atopic dermatitis (105), whereas in psoriasis strong expression of E-selectin was detected on vascular endothelial cells (10), correlating with the severity of pruritus. In addition, an increased serum concentration of soluble vascular adhesion protein (VAP)-1 was found in psoriasis subjects with pruritus compared to patients free from this symptom (106). Unfortunately, there are currently only scarce data as regards the possible role of ves-

sels and adhesion molecules, and this direction of investigation deserves interest in the future.

Free radicals (ROS)

Reactive oxygen species (ROS) could also constitute a valuable field for further exploration as regards mediation of pruritus. The role of free radicals in modulating the neurogenic vascular response and thermal hyperalgesia in rats with chronic constrictive nerve injury (CCI) was demonstrated (107) and free radicals, *via* interaction with NO to form peroxynitrite, have been implicated in the maintenance of thermal hyperalgesia in these rats. Further, CCI rats had a significantly higher xanthine oxidase activity in the injured sciatic nerve. It was widely demonstrated (36,37) that intradermal SP increased NO in the skin, and that NO enhanced SP-induced itch-associated responses. In mice, it was observed that NO production was markedly increased in mainly scratched areas compared to non-scratched regions (108). Further, the itch associated response was significantly suppressed by i.v. injection of NO synthase (NOS) inhibitor. As regards the possible mechanism of action, it seems that NO synergistically potentiates IL-1 β -induced increase of COX-2 mRNA levels, resulting in the facilitation of SP release from primary afferent neurons (109). Inducible NOS (iNOS) expression is not found in most resting cells (110). Exposure to proinflammatory cytokines such as IL-1, TNF- α or IFN- γ induces the expression of iNOS gene in various inflammatory and tissue cells. Many mouse cells readily express iNOS in response to a single cytokine, whereas human cells usually require a combination of different cytokines for detectable iNOS expression and NO synthesis. For instance, IFN- γ is an important cytokine for iNOS expression in human cells as in murine cells (110). Hence, in an intent to elucidate how ultraviolet B rays (UVBR) regulate iNOS expression in skin under inflammation conditions in cultured murine keratinocyte Pam 212 cells (111) it was observed that low doses of UVBR significantly suppressed IFN- γ or TNF- α -induced NO production and iNOS expression at both the mRNA level and the protein level.

Other mediators

Pruritus may be elucidated by the opioid system as well. It is believed that activation of μ -opioid receptors induces while activation of κ -opioid receptors alleviates pruritus (112). Studies have demonstrated that epidermal opioid systems are associated with the modulation of pruritus in atop-

ic dermatitis (113) but also in human hypertrophic scars (114). It was shown that both naloxone and naltrexone, the potent μ -opioid receptor antagonists, reduced histamine-induced pruritus in atopic dermatitis subjects to a greater extent than antihistaminic drugs (115,116) and on the other hand, k -opioid receptor agonists like nalfurafine or butorphanol led to significant reduction of itching both in humans (117) and in primates (118).

HOW COULD TOPICAL SUPEROXIDE DISMUTASE (SOD) ALLEVIATE PRURITUS?

In humans, as in all mammals and most chordates, three forms of SOD are present, i.e. SOD1 is located in the cytoplasm, SOD2 in the mitochondria and SOD3 is extracellular. The first is a dimer (consisting of two units), while the others are tetramers (4 subunits). SOD1 and SOD contain copper and zinc, while SOD2 has manganese in its reactive center. The enzyme SOD catalyzes dismutation of superoxide into oxygen and hydrogen peroxide. SOD outcompetes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. SOD is used in cosmetic products to reduce free radical damage to the skin, for example to reduce fibrosis following radiation for breast cancer. SOD is known to reverse fibrosis perhaps through reversion of myofibroblasts back to fibroblasts.

In our daily practice, on many occasions we were able to observe that topical SOD seemed to possess a strong antipruritic activity, even in antihistaminic-resistant pruritus. Then we decided to investigate the levels at which SOD could act, in order to explain this antipruritic activity observed. There are no literature data permitting to suspect any activity or interference of SOD with H1- or H4-receptors, or with PAR-2, or with any other receptors involved in pruritus.

On the contrary, a lot of possible interferences of SOD with numerous mediators of pruritus can be detected from the published data. Interestingly, in neurogenic plasma exudation enhanced by SP in guinea pig lungs, SOD was found to significantly inhibit this neurogenic plasma leakage, suspecting a possible antagonist effect of SOD on NK-1 receptors (36). In rats with chronic constriction nerve injury, the neurogenic vascular response was significantly improved in a similar manner by the treatment with SOD (107). It is of great relevance to note that SOD was shown to clearly decrease the CGRP mRNA expression in a rat

model of ischemic facial paralysis (119), strongly suggesting a regulatory role of SOD on CGRP.

As regards neurotrophins, there are no data on a direct influence of SOD on NGF, but it is obvious and we shall later review it, that the levels of NGF being up-regulated by various proinflammatory cytokines (63) and ROS, will be indirectly down-regulated by the effect of SOD on these factors; the down-regulating effect of SOD on a great number of cytokines is now well-known; let us focus on the major cytokines involved in pruritus.

The first key player is TNF- α , and there is a lot of evidence that SOD is also capable of reducing the levels of TNF- α in lung macrophages (45,120), spleen cells (121), whole blood (122) or endothelial cells (123,124). As regards interleukins, we have pointed out the major role played by IL-1 in the pathogenesis of pruritus. Meanwhile, there is a bulk of literature assessing the down-regulation of IL-1 induction by SOD, in vascular smooth muscle cells (125), lung tissue (124,126) or microglial cells (126). IL-2 is another interleukin involved in the mechanism of pruritus. *In vivo* administration of SOD was found to reduce IL-2 production, to suppress IL-2 receptor expression and to depress IL-2 mediated lymphocyte proliferation response after trauma (127). A similar down-regulating activity of SOD was shown on IL-2 expression in Jurkat T cells (128). Another player, IL-6, is also under influence of SOD. The release of IL-6 is significantly decreased in lung tissue in the presence of SOD (122,124), but also in myocytes (129), microglial cells (126) or skin cells (130). As regards IL-31, there are currently no data in the literature, probably due to the recent discovery of this new cytokine. Vessels and adhesion molecules have also been implicated in the pathogenesis of pruritus, in particular E-selectin. There is a great body of evidence that SOD reduces the levels of E-selectin in both lung tissue (131) and endothelial cells (132-134). The interaction between SOD and ROS is a well-documented issue, especially with NO. NO production and iNOS mRNA expression in response to IL-1 stimulation, was significantly down-regulated in chondrocytes by pretreatment with tat-SOD fusion proteins (135). In vascular wall, augmented iNOS expression was reduced by EC-SOD treatment (136). In gastric and ileal mucosa, treatment with PEG-SOD or PEG-CAT was able to diminish the presence of nitrotyrosine, and reduce the LPS-induced increase of iNOS positive residential macrophages (137).

It was also demonstrated that iNOS expressing brain tumor cells protected themselves against

NO toxicity by over-inducing SOD (138). In embryo chorioallantoic membrane SOD was also found to down-regulate iNOS expression and NO production in a dose-dependent manner (139).

In the lung, four hours after LPS administration, mRNA expression of iNOS, IL-1 β and MnSOD was significantly enhanced, while SOD, CAT and SOD + CAT reduced the quantity of exhaled NO and plasma nitrate concentration, attenuating LPS-induced oxygen radical release (140). Transgenic CuZn-SOD also inhibited NO synthase induction in experimental subarachnoid hemorrhage (141). In hepatocytes, NO release and nitrotyrosine expression induced by activated macrophages was successfully attenuated by SOD (142).

Treatment of astrocyte cultures for 18 h with LPS and IFN- γ produced a dose-dependent increase of iNOS and this effect was significantly decreased by the addition of SOD/CAT in the medium (143). It seems that antioxidant enzymes suppress NO production through the inhibition of NF-kappa B activation (144). A nitric oxide-releasing agent, S-nitroso-N-acetylpenicillamine, was shown to enhance the expression of SODmRNA in the murine macrophage cell line RAW264-7, suggesting that when producing NO, RAW 264-7 cells express SOD that might protect them from NO toxicity (72).

All these data taken together strongly demonstrate that there is an unquestionable efficacy of SOD in reducing or even suppressing NO production and diminishing iNOS expression in various cell types and under various circumstances.

To summarize all these data regarding the anti-pruritic activity of SOD, it can be stated that:

- there is an possible antagonist effect of SOD on NK-1 receptors (36);
- the levels of NGF can be indirectly down-regulated by SOD through its activity on various proinflammatory cytokines (63,92);
- there is clearly a regulatory role of SOD on CGRP production and expression (119);
- although not acting directly on SP, SOD is considerably attenuating its effects by down-regulating TNF- α , IL-1, IL-6 and NO, whose secretion is stimulated by SP;
- SOD is obviously recognized as being capable of reducing the emission and expression of numerous cytokines, many of them (TNF- α , IL-1, IL-2, IL-6, E-selectin) having a definitely proven role in the pathogenesis of pruritus; and
- there is an obvious efficacy of SOD in reducing

or even suppressing NO production and diminishing iNOS expression, being established that NO has a key role in the occurrence and maintenance of pruritus.

CONCLUSION

This brief review of recent frontiers in the knowledge of pruritus reveals that we still lack a single, unique, universally effective and accepted pharmacological tool to combat itch and pruritus, and due to the inherent neurophysiologic and neuroimmunologic complexity of itch pathophysiology, it would be naive to expect future advent of such a miraculous drug.

Hence, any new opportunity in the armamentarium to combat pruritus is welcome, and it seems that the previous (unpublished) encouraging results obtained with topical SOD could have some scientific basis to explain its alleged efficacy.

Nevertheless, there is still a long way to go, and there is an obvious need for more investigations, more precisely focused on the own interference of SOD in the mechanisms of pruritus.

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