

Over-expression of Mn-superoxide dismutase as a marker of oxidative stress in lesional skin of chronic idiopathic urticaria

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Summary

We studied the involvement of oxidative stress in chronic idiopathic urticaria (CIU), assessing the activities of superoxide dismutase (SOD) and glutathione and the levels of malondialdehyde (MDA), a marker of lipid peroxidation, in samples taken from lesional skin ($n = 16$) and nonlesional skin ($n = 11$) of CIU patients. The activity of SOD and glutathione and the levels of MDA were markedly increased in lesional skin as compared with skin of healthy subjects, whereas no differences were detected between nonlesional skin of CIU patients and control samples. Immuno-dot blot assay revealed an up-regulation of Mn-SOD expression in lesional skin. These findings show that oxidative stress is crucially involved in CIU. The evidence of lipid peroxidation and compensatory increase of Mn-SOD and glutathione activities in lesional skin, in the absence of any alteration in uninvolved skin, suggests that oxidative stress is secondary to the development of inflammation.

Report

The production of reactive oxygen species (ROS) and disturbance in redox status can modulate a great variety of cellular processes, including signal transduction, gene expression, inflammation and apoptosis, and is implicated in the pathogenesis of several cutaneous inflammatory disorders,^{1,2} including physical urticarias.³ Superoxide anion, a free radical generated during the monovalent metabolic reduction of oxygen, is toxic to biological systems. There are several pathways in aerobic cells that can lead to the production of superoxide anion. Mitochondria and endoplasmic reticulum have been shown to produce superoxide anion as a consequence of auto-oxidation of electron transport chain components. Other sources of superoxide anion are cytosolic enzymes, such as xanthine oxidase, and neutrophils.

The purpose of our research was to evaluate the involvement of oxidative stress in chronic idiopathic

urticaria (CIU), measuring malondialdehyde (MDA), a marker of lipid peroxidation, and the activity of the antioxidants superoxide dismutase (SOD) and glutathione, in both lesional and nonlesional skin.

Sixteen patients with CIU, nine females and seven males with a mean age of 41 years (range: 20–63 years) were selected. Their urticaria was characterized by at least three episodes per week for more than 6 weeks. The association with physical and cholinergic urticarias, urticarial vasculitis, infectious foci, food and drug allergies and any relevant systemic disorder was excluded. A 5-mm punch biopsy was taken from spontaneous wheals of variable duration, from 30 min to 4 h (timed on the basis of patient's estimation). For noninvolved CIU control biopsies 11 patients in whom no wheals had developed on uninvolved skin during the previous 48 h were selected. Any active treatment was discontinued at least 2 weeks prior to the biopsy, with the exception of corticosteroids, immunosuppressants and astemizole, which were interrupted for a minimum of 6 weeks. Skin samples obtained from 10 healthy volunteers were used as non-CIU controls. Skin samples were homogenized and sonicated in order to obtain soluble material and protein aliquots of 1 and 2 mg were used for the

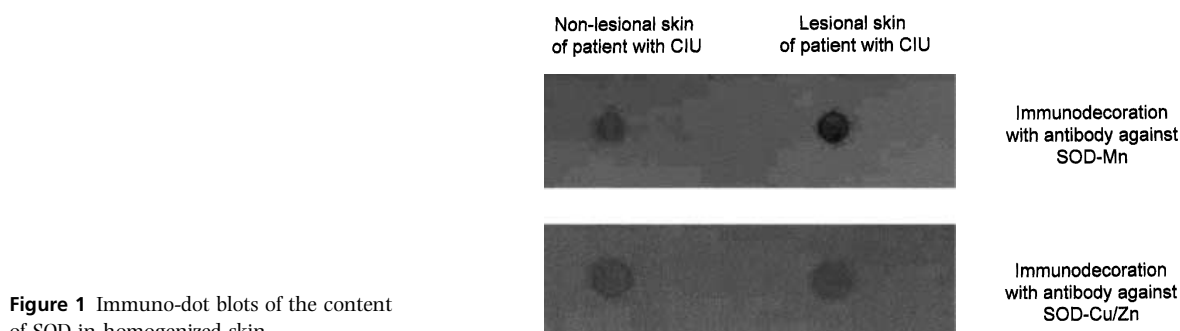
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Table 1 Activity of superoxide dismutase and glutathione, and levels of malondialdehyde in skin of patients and controls.

	SOD (U/mg protein/min) mean value (range)	Glutathione (U/mg protein/min) mean value (range)	MDA (μ mol/L) mean value (range)
Lesional skin of patients with CIU (<i>n</i> = 16)	43.3 (33.6–60.2)	38.4 (31.8–45.1)	3.1 (2.4–4.2)
Non-lesional skin of patients with CIU (<i>n</i> = 11)	11.6 (10.4–13.2)	16.8 (15.3–19.7)	0.7 (0.6–0.9)
Control samples (<i>n</i> = 10)	11.8 (10.0–13.5)	17.1 (15.5–20.0)	0.7 (0.6–0.9)

The homogenized skin samples were diluted at the concentration of 1 mg/ml in lysis buffer. Each sample was spotted on nitrocellulose after filtration under vacuum and probed with antibodies against SOD-Mn or SOD-Cu/Zn as reported in¹¹.

**Figure 1** Immuno-dot blots of the content of SOD in homogenized skin.

measurement of enzymatic activity. SOD activity was determined by means of the xanthine-xanthine oxidase system,⁴ MDA was assessed using thiobarbiturate method,⁵ and a colourimetric reaction using 5,5'-dithiobis-(2-nitrobenzoate) as chromogen was used for glutathione assay.⁶ Statistical analysis was performed using Wilcoxon's test to compare the results between lesional and nonlesional skin of patients with CIU, and Mann-Whitney *U*-test to evaluate differences between skin of patients and that of the controls. *P* values < 0.05 were considered significant.

The activity of SOD and glutathione and the levels of MDA were notably increased in all samples from lesional skin as compared with unaffected skin of patients (*P* < 0.005) and control samples (*P* < 0.001), whereas no notable differences were noted between uninvolved skin of CIU patients and healthy control skin (Table 1).

In lesional skin of our patients we found an over-expression of Mn-SOD (immuno-dot blot assay, Bio-Rad Laboratories, Segrate, Milan, Italy), which was in good correlation with the increase of enzymatic activity. In contrast, in the same skin samples the amount of Cu,Zn-SOD was similar to that observed in the skin of healthy subjects (Fig. 1).

In light of these results, it can be hypothesized that oxidative stress is a crucial event in CIU. The evidence of lipid peroxidation and compensatory increase of SOD and

glutathione activities in lesional skin, in association with normal findings in uninvolved skin, supports the suggestion that oxidative stress is linked to inflammation and may be a consequence of the activation of the infiltrating cells, such as neutrophils and eosinophils. Interestingly, *in vitro* studies⁷ indicate that human basophils and rat mast cells can generate superoxide in response to challenge with antigen or with anti-Fc ϵ RI antibody. In turn, ROS can have a notable influence on the activation and degranulation of basophils and mast cells.^{7–9}

It is well-known that SOD exerts an important protective action, catalysing the dismutation of the superoxide anion radical into hydrogen peroxide, and includes three forms, two of which are intracellular (Cu,Zn-SOD in the cytosol and Mn-SOD in mitochondria). The up-regulation of Mn-SOD gene expression in CIU may represent a defence mechanism against the ROS generated by the respiratory chain complexes of the inner mitochondrial membrane such as cytochrome *c* oxidase. It is likely that the over-expression of Mn-SOD results from an increased secretion of cytokines, as reported in psoriasis.¹⁰

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