

Markers of antioxidant defence system and lipid peroxidation in peripheral blood of female patients with chronic idiopathic urticaria

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Abstract Oxidative stress is an important event in lesional skin of patients with chronic idiopathic urticaria (CIU). In the present study, we assessed blood oxidant/antioxidant status of patients suffering from CIU with positive response to autologous serum skin test (ASST) and with negative ASST, to improve our understanding of biological processes and the part of oxidative stress in this disease. Activities of manganese superoxide dismutase (MnSOD), copper–zinc superoxide dismutase (Cu/ZnSOD), glutathione peroxidase (GSH-PX), and catalase (CAT) as indices of enzymatic antioxidant capacity, as well as malondialdehyde (MDA) level as a maker of lipid peroxidation were measured in plasma and erythrocytes from 14 CIU female patients showing positive ASST, 31 CIU female patients with negative ASST and in 19 sex- and age-matched healthy subjects. The antioxidant enzyme activity in plasma and in erythrocytes did not differ significantly among the three groups. Also, the plasma and erythrocytes MDA levels were similar in the three groups. Based on our results, it seems that systemic activity of the enzymatic antioxidants (CuZn/SOD,

MnSOD, GSH-Px, and CAT) as well as level of lipid peroxidation determined by MDA may not be increased in the course of immune-inflammatory processes associated with CIU. We also suggest that the systemic oxidant/antioxidant status of CIU patients, showing positive response to ASST, may not be different from that of CIU patients with negative ASST.

Keywords Oxidative stress · Lipid peroxidation · Antioxidant enzyme activity · Chronic idiopathic urticaria

Introduction

The origin of chronic urticaria has been, to a great extent, unknown; yet, in some cases autoimmune aetiology is proposed [6, 15]. There is increasing evidence to prove that some skin diseases, including urticaria, are mediated by oxidative stress [9], which is caused by imbalance between production of highly reactive oxygen species (ROS) and endogenous protective enzymatic and non-enzymatic antioxidative capacity [13]. ROS are generated during normal metabolism and are integral parts of normal cellular function [9]; they exert multiple effects, including oxidative damage to DNA, leading to various human disorders and among these, the autoimmune diseases [2]. Interestingly, it has been demonstrated that mast cell and basophiles are capable of generating superoxide anion, which may regulate biological processes, including regulation of mast cells and basophiles activation and mediators release under both physiological and pathological conditions [17].

Local and systemic changes in enzymatic antioxidant defence system and intensification of lipid peroxidation

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are associated with different types of immune and inflammatory processes. ROS-mediated membrane lipid peroxidation results in production of malondialdehyde (MDA)—a highly toxic molecule, which is used to determine the degree of lipid peroxidation and as a biological marker of oxidative stress [5]. Antioxidant enzymes, such as manganese superoxide dismutase (MnSOD), copper–zinc superoxide dismutase (Cu/ZnSOD), glutathione peroxidase (GSH-Px), and catalase (CAT) act in a cooperative or synergistic manner to ensure cell protection against oxidative stress. Their optimal effect, as scavengers of ROS, is achieved only if appropriate balance between activities of these enzymes is maintained [7]. The activity of antioxidant enzymes and the level of lipid peroxidation endproduct were markedly increased in lesional skin of patients with chronic idiopathic urticaria (CIU), as compared with skin of the healthy subjects. Moreover radical oxygen species production and plasma SOD activity were increased in CIU patients, suggesting that oxidative stress is a crucial event in this disease and proves secondary to the development of inflammation [4, 12]. In the present study, we investigated indirect parameters of ROS activity in plasma and erythrocytes of patients suffering from CIU, to determine whether there were any differences in the systemic oxidative/antioxidative status of CIU patients in the presence or absence of positive response to autologous serum skin test (ASST). The following indices of oxidant/antioxidant system activity were determined: activity of a variety of antioxidative enzymes such as MnSOD, Cu/ZnSOD, GSH-Px, and CAT, as well as the level of MDA, lipid peroxidation endproduct.

Patients and methods

The study group consisted of 45 non-atopic and non-smoking symptomatic women with active CIU in whom all identified causes of the diseases had been excluded. Those with physical urticaria were not included in the study. The patients had not taken steroids for longer than 12 months and antihistamines for at least 10 days preceding the study. CIU patients were divided into two groups, depending on the presence or absence positive response to ASST. Thirty-one patients (median age 31 years, interquartile range 27–34) showed negative wheal response to intradermal injection of autologous serum (negative-ASST CIU group) and 14 (median age 33 years, interquartile range 28–33) proved positive response to ASST (positive-ASST CIU group). The disease intensity was measured by assessment of wheals and pruritus on a 0–3 scale sum of score 0–6 proposed by Zuberbier et al.

[18]. Briefly, the number of the wheals and severity of pruritus were scored as follows: number of wheals, 0 = no wheals, 1 = mild wheals (<20 wheals/24 h), 2 = moderate wheals (21–50 wheals/24 h), 3 = intense wheals (>50 wheals/24 h, or large confluent areas of wheals); pruritus, 0 = none, 1 = mild, 2 = moderate, 3 = intense. Our patients' scoring was from 3 to 6.

The control group consisted of 19 non-atopic, non-smoking healthy women (median age 30 years, interquartile range 28–33) with negative response to ASST.

All participants gave written, informed consent, and the study was approved by the University Ethics Committee.

Blood sampling

Venous whole blood samples (heparinized) were collected between 07:00 and 08:00 a.m. after 12 h fasting state, and separated by centrifugation (at 1,000×g for 15 min at 4°C) into plasma and erythrocytes. Erythrocytes were washed three times with 0.9% NaCl, then lysed in distilled water.

Biochemical procedures

The activities of superoxide dismutases (MnSOD and Cu/ZnSOD) in plasma and/or erythrocyte were estimated by Oyanagui method [10]. The activity of SOD is expressed in nitrite units (NU) per ml and g Hb (NU/ml and NU/g Hb), respectively.

Erythrocyte glutathione peroxidase activity (GSH-Px) was estimated using the method by Paglia and Valentine [11]. The enzyme activity is presented in μmol NADPH consumed per minute and per gram Hb (μmol NADPH/g Hb/min).

Catalase (Cat) activity in erythrocyte was determined according to the method by Aebi et al. [1]. The results are expressed as U/mg Hb.

Malondialdehyde (MDA) levels in plasma and erythrocyte were determined according to Ohkawa et al. [8], using reaction with thiobarbituric acid, and calculated by a spectrophotometer. The results are expressed in $\mu\text{mol/l}$ and $\mu\text{mol/g}$ Hb, respectively.

Skin prick tests

Allergic status was evaluated by skin prick testing using a panel of common inhalant allergens and main food allergens (Allergopharma, Reinbek, Germany). Saline and histamine solutions were used as negative and positive controls, respectively. Wheal diameter greater by 3 mm than the negative control was accepted as a positive response.

Autologous serum skin test (ASST)

Intradermal tests with patient's own serum were performed according to the method described by Sabroe et al. [14]. A serum-induced wheal of diameter greater by at least 1.5 mm than that of a control wheal induced with physiological saline was accepted as positive.

Statistical analysis

The data are expressed as medians and interquartile ranges. Parameters were tested for normal distribution using Shapiro–Wilk test. Variance analysis was evaluated by means of the Levene test. The Mann–Whitney *U* test was used to compare results from urticaria patients (as a whole) and controls. The Kruskal–Wallis test was used for screening differences between the three groups. *P* values lower than 0.05 were considered statistically significant.

Results

The antioxidative enzyme activity in plasma (CuZn/SOD and MnSOD) and in erythrocytes (CuZn/SOD, GSH-Px, and CAT) as well as plasma and erythrocyte level of MDA did not differ significantly between CIU patients and the healthy subjects (data not included).

Table 1 shows the parameters of the oxidative/antioxidative system in the control group, the positive-

ASST/CIU group as well as the negative-ASST/CIU group. No significant differences were observed among the three groups for all the parameters analysed.

Discussion

In our study, the lipid peroxidation product, plasma and erythrocyte level of MDA in CIU patients were not statistically different from those of the healthy controls. Moreover, activity of the scavenging enzymes, measured in plasma (CuZn/SOD and MnSOD) and erythrocytes (CuZn/SOD, GSH-Px, and CAT) did not show any significant differences between CIU patients and the controls. Taken together, it seems that significant changes in activity of enzymatic antioxidant defence system as well as enhanced activity of ROS, resulting in increased membrane lipid peroxidation, may not occur in the peripheral blood of CIU patients.

So far, only few studies have focused on the part of oxidative stress in urticaria. The alteration in oxidant/antioxidant homeostasis has been reported in patients showing some forms of physical urticaria. The occurrence of systemic imbalance between peroxidable compounds and the antioxidant system in patients with cold and solar urticaria has been demonstrated [3]. Probably the imbalance reflects the alteration of cytokine production or activation of a complement pathway and could be a common biochemical basis for increased skin susceptibility to external stimuli in

Table 1 SOD, GSH-Px activity and MDA levels in erythrocyte and plasma of CU women with positive or negative response to ASST and in the healthy controls

Analysed parameters (unit)	Control group (<i>n</i> = 19) Median interquartile ranges	Positive-ASST/CIU group (<i>n</i> = 14) median interquartile ranges	Negative-ASST/CIU group (<i>n</i> = 31) median interquartile ranges
Erythrocyte			
CuZnSOD (U/g Hb)	134.4 81.5–181.0	130.5 98.2–181.9	142.0 89.9–166.1
GSH-Px (μmol/NADPH/g Hb/min)	40.3 20.9–54.6	51.6 28.0–57.9	40.0 31.7–54.0
MDA (μmol/g Hb)	0.33 0.28–0.39	0.34 0.29–0.36	0.33 0.29–0.43
CAT (U/mg Hb)	405.57 377.7–470.9	458.4 424.2–480.0	441.4 414.6–463.6
Plasma			
CuZnSOD (NU/ml)	13.4 11.9–16.0	13.5 10.7–14.9	14.4 13.1–17.3
MnSOD (NU/ml)	7.5 3.9–11.4	6.8 4.8–7.4	7.6 5.8–8.9
MDA (μmol/l)	1.8 1.6–2.4	2.3 1.9–2.7	2.0 1.6–2.3

CU chronic urticaria, ASST autologous serum skin test, CAT catalase, Cu/ZnSOD copper–zinc superoxide dismutase, GSH-Px glutathione peroxidase, MDA malondialdehyde, MnSOD manganese superoxide dismutase

physical urticaria patients [3]. The activity of MnSOD and glutathione, and the level of MDA were increased markedly in lesional skin of CIU patients as compared with skin of the healthy subjects [12]. On the contrary, the same skin samples showed CuZn-SOD activity similar to that observed in the skin of healthy subjects. In addition, no differences were detected between uninvolved skin from CIU patients and the healthy controls. The results suggest that oxidative stress is a crucial event in CIU, associated with inflammation and may be the consequence of various cells activation upon urticarial processes [12]. Moreover, it has been reported that production of ROS and plasma SOD activity was significantly elevated in peripheral blood of patients with CIU at baseline as compared to control subjects and treatment with desloratadine caused relevant reduction of oxidative stress parameters [4]. On the contrary, we did not observe any significant differences between CIU patients and the healthy controls in SOD activity, nor in other antioxidative enzymes determined in plasma and erythrocytes.

Interestingly, the balance of oxidative/antioxidative influences may play an important role in modulation of mast cells function, including their mediators' release, especially in the context of experimental autoimmunity [16]. On the basis of ASST results, our study distinguished between two groups of CIU patients. Such tests have been extensively used in clinical practice where positive response suggests the autoimmune basis of the disease [14, 15]. The indirect parameters of ROS activity, we investigated, were similar in the negative-ASST CIU subgroup and the positive-ASST CIU subgroup, suggesting that the oxidant/antioxidant profiles of such patients do not differ.

As indicated by the data earlier, there are some differences in the oxidative/antioxidative status assessed in samples of lesional skin, uninvolved skin, and peripheral blood of patients suffering from CIU, as compared with the healthy subjects.

Taken together, in our study, the behaviour of the investigated indirect parameters of reactive oxygen species activity in peripheral blood did not differ significantly between CIU patients and the healthy controls. It seems that systemic changes in the activity of enzymatic antioxidants (CuZn/SOD and MnSO, GSH-Px, and CAT) and the level of lipid peroxidation measured by MDA levels may not occur in the course of CIU associated processes. We also suggest that the systemic oxidant/antioxidant status of CIU patients, showing positive response to ASST, may not be different from that of CIU patients with negative ASST.

However, definite assessment of the behaviour of antioxidant defence system in urticaria patients and determination of oxidative stress significance in this disease, demands investigation of other components of the oxidant/antioxidant system, including non-enzymatic antioxidants, in patients showing different forms of this disease.

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