

# Antioxidant Enzyme Activity and Malondialdehyde Concentration in the Plasma and Erythrocytes of Patients With Urticaria Induced by Nonsteroidal Anti-inflammatory Drugs

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## ■ Abstract

*Background:* It has been suggested that oxidative stress is a crucial event in some forms of urticaria.

*Aim:* To evaluate the blood oxidant/antioxidant profile of patients suffering from urticaria induced by nonsteroidal anti-inflammatory drugs (NSAIDs).

*Methods:* We measured the activity of the antioxidant enzymes copper-zinc superoxide dismutase (Cu/ZnSOD), glutathione peroxidase (GSH-Px), and catalase (CAT), and the levels of malondialdehyde (a marker of lipid peroxidation) in the plasma and erythrocytes of 12 females with NSAID-induced urticaria and in 19 healthy controls.

*Results:* The enzyme activity in plasma (Cu/ZnSOD) and in erythrocytes (Cu/ZnSOD, GSH-Px, and CAT) did not differ significantly between urticaria patients and controls. Moreover, the levels of malondialdehyde in plasma and erythrocytes did not differ significantly between the 2 groups.

*Conclusions:* It seems that processes associated with urticaria induced by NSAIDs may not modify antioxidant enzyme activity and may not enhance lipid peroxidation in peripheral blood.

**Key words:** Lipid peroxidation. Antioxidant enzyme activity. Urticaria. Nonsteroidal anti-inflammatory drug intolerance.

## ■ Resumen

*Antecedentes:* Se ha sugerido que el estrés oxidativo es un episodio clave en algunas formas de urticaria.

*Objetivo:* El objetivo fue evaluar el perfil de oxidantes/antioxidantes de la sangre en pacientes que padecen urticaria inducida por antiinflamatorios no esteroideos (AINE).

*Métodos:* Realizamos una medición de la actividad de las enzimas antioxidantes superóxido dismutasa de cobre-zinc (Cu/ZnSOD), glutatión peroxidasa (GSH-PX) y catalasa (CAT) y las concentraciones de malondialdehído (un marcador de la peroxidación lipídica), en el plasma y los eritrocitos de 12 mujeres con urticaria inducida por AINE y de 19 controles sanos.

*Resultados:* La actividad enzimática en el plasma (Cu/ZnSOD) y en los eritrocitos (Cu/ZnSOD, GSH-Px y CAT) no difirió de manera significativa entre los pacientes con urticaria y los controles. Además, las concentraciones de malondialdehído en el plasma y en los eritrocitos, tampoco difirieron significativamente entre los 2 grupos.

*Conclusiones:* Parece que los procesos asociados con la urticaria inducida por AINE no modifican la actividad enzimática antioxidante y no aumentan la peroxidación lipídica en la sangre periférica.

**Palabras clave:** Peroxidación lipídica. Actividad enzimática antioxidante. Urticaria. Intolerancia a los fármacos antiinflamatorios no esteroideos.

## Introduction

Oxygen free radicals, such as superoxide anion radical, singlet oxygen, hydroxyl radical, and perhydroxyl radical are together referred to as reactive oxygen species (ROS) and play an important role in the pathogenesis of several diseases [1], including some skin diseases [2,3]. Malondialdehyde (MDA), the end product of lipid peroxidation, is a good marker of free radical-mediated damage and oxidative stress [4]. Antioxidant enzymes such as copper-zinc superoxide dismutase (Cu/ZnSOD), glutathione peroxidase (GSH-Px), and catalase (CAT) protect cells against oxidative stress [5].

Interestingly, it has been suggested that oxidative stress is a crucial event in some forms of urticaria, including chronic idiopathic urticaria (CIU) [6] and physical urticaria [7]. Moreover, it has been hypothesized that oxidative stress induced by nonsteroidal anti-inflammatory drugs (NSAIDs) initiates cytotoxic processes leading to adverse effects [8,9]. Hypersensitivity to NSAIDs, including aspirin, can cause acute urticaria or aggravate pre-existing chronic urticaria [10]. Systemic changes in antioxidant enzyme activity and lipid peroxidation have been demonstrated in patients with physical urticaria [7], but not in patients with CIU [11]. Unfortunately, oxidative stress in other forms of urticaria has not been examined in the literature. Therefore, we assessed some parameters of lipid peroxidation and enzymatic antioxidant status of patients with urticaria induced by NSAIDs. The indirect parameters of ROS activity determined were enzyme activity in plasma (CuZn/SOD) and in erythrocytes (CuZn/SOD, GSH-Px, and CAT), as well as plasma and erythrocyte levels of MDA.

## Material and Methods

The study included 12 symptomatic female patients (median age, 33 years, interquartile range [IQR], 31-36 years)

with urticarial skin lesions, with or without angioedema, caused by NSAIDs (acetylsalicylic acid, diclofenac, ibuprofen, ketoprofen, naproxen). The patients had no history of chronic urticaria or acute urticarial symptoms caused by other factors. Eight of the 12 patients had a clear clinical history of at least 2 events within 3 hours after ingestion of the drugs. Four of the 12 patients reported only 1 urticarial event after NSAIDs, and this was confirmed by oral challenge test with acetylsalicylic acid. Patients had no history of asthma or anaphylaxis (the clinical characteristics of patients are presented in Table 1). Blood for analysis was taken within 24 hours from the onset of symptoms. The patients had not taken anti-urticaria drugs. The control group consisted of 19 healthy nonsmoking women (median age, 30 years [28-33]) [11].

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

### Biochemical Procedures

The activity of Cu/ZnSOD in plasma and erythrocytes were assessed according to Oyanagui [12]. The values are expressed in nitrite units (NU) per mL and g Hb (NU/mL and NU/g Hb), respectively.

The activity of GSH-Px in erythrocytes was estimated using the method described by Paglia and Valentine [13]. The values are presented in  $\mu\text{mol}$  of nicotinamide adenine dinucleotide phosphate consumed per minute and per g Hb.

The activity of CAT in erythrocytes was measured using the method by Aebi et al [14]. The results are expressed as U/mg Hb.

The levels of MDA in plasma and erythrocytes were determined according to Ohkawa et al [15]. The results are presented in  $\mu\text{mol/L}$  and  $\mu\text{mol/g}$  Hb, respectively.

### Skin prick tests

Skin prick testing was performed using a panel of common inhalant allergens (grass, weed and tree pollens, cat

Table 1. Clinical Characteristics of Patients With NSAID-Induced Urticaria

Patient	NSAIDs Intolerance	Challenge Test With ASA	Atopy	Comorbid Disorders
1	ASA, ibuprofen	NP	+	None
2	Diclofenac, ibuprofen	NP	+	None
3	Naproxen	Positive	-	Chronic rhinosinusitis
4	Ketoprofen, naproxen	NP	+	Seasonal allergic rhinitis
5	Ketoprofen	Positive	-	Nasal polyps
6	Unknown NSAID	Positive	-	Nasal polyps
7	Naproxen, ibuprofen	NP	-	None
8	ASA, ketoprofen	NP	-	None
9	ASA	Positive	-	Chronic rhinosinusitis
10	Ketoprofen, naproxen, ASA	NP	-	None
11	Diclofenac, ASA	NP	+	None
12	Ibuprofen, ketoprofen	NP	+	None

Abbreviations: ASA, acetylsalicylic acid; NP, not performed; NSAID, nonsteroidal anti-inflammatory drug.

and dog dander, molds, *Dermathophagoides pteronyssinus*, and *Dermathophagoides farinae* (Allergopharma, Reinbek, Germany). Saline and histamine solutions were used as negative and positive controls, respectively. A wheal with a diameter that was 3 mm wider than the negative control was accepted as a positive response.

Atopy was defined as a skin prick test result that was positive to at least 1 of the aeroallergens.

### Statistical Analysis

Data are expressed as the median (IQR). Comparison between the groups was by the Mann-Whitney unpaired rank sum test. *P* values lower than .05 were considered statistically significant.

## Results

Table 2 shows the parameters of the oxidative/antioxidative system in the urticaria group and the control group.

Table 2. Activities of Copper-zinc Superoxide Dismutase (Cu/ZnSOD), Glutathione Peroxidase (GSH-Px), and Catalase (CAT) as Well as Malondialdehyde (MDA) Level in the Peripheral Blood of Healthy Controls and Patients With Nonsteroidal Anti-Inflammatory-Drug-Induced Urticaria<sup>a</sup>

Measure Indices (unit)	Control Group	Urticaria Patients
Erythrocyte CuZnSOD (U/g Hb)	134.4 (81.5-181.0)	138.8 (103.4-192.0)
GSH-Px (μmol/NADPH/g Hb/min)	40.3 (20.9-54.6)	45.7 (25.8-62.2)
MDA (μmol/g Hb)	0.33 (0.28-0.39)	0.4 (0.32-0.42)
CAT (U/mg Hb)	405.6 (377.7-470.9)	425.5 (364.8-452.0)
Plasma CuZnSOD (NU/mL)	13.4 (11.9-16.0)	12.3 (9.8-15.2)
MDA (μmol/L)	1.8 (1.6-2.4)	1.94 (1.45-2.5)

<sup>a</sup> Values are expressed as the median (interquartile range).

Abbreviations: CAT, catalase; CuZnSOD, copper-zinc superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; NSAID, nonsteroidal anti-inflammatory drug.

The activity of CuZn/SOD in plasma and the activity of CuZn/SOD, GSH-Px, and CAT in erythrocytes did not differ significantly between the urticaria group and the healthy control group, as described elsewhere [11]. The same was true for plasma and erythrocyte MDA levels.

## Discussion

It has been suggested that NSAIDs have different influences on oxidative stress and antioxidant-related parameters in vivo [16]. We did not observe any significant changes in lipid peroxidation expressed as the MDA level in patients suffering from urticaria caused by NSAIDs as compared with healthy controls. Moreover, no significant changes were observed in enzymatic antioxidant defense measured by the activity of CuZn/SOD in plasma and the activity of CuZn/SOD, GSH-Px, and CAT in erythrocytes between the patients with NSAID-induced urticaria and the controls. Few studies to date have examined the significance of oxidative stress in urticaria. Urticarial processes have been associated with oxidative stress at the site of inflammation, as have some systemic changes in indices of ROS metabolism [6,7]. The activity of some antioxidant enzymes and the level of MDA were markedly higher in the lesional skin of patients with CIU than in the skin of the healthy controls [6]. However, in our study, lipid peroxidation product, plasma, and erythrocyte levels of MDA were no higher in patients than in controls. Moreover, the activity of scavenging enzymes, measured in plasma (CuZn/SOD and manganese SOD) and erythrocytes (CuZn/SOD, GSH-Px, and CAT) showed nonsignificant differences between CIU patients and the controls [11].

These data point to differences in the behavior of indices of oxidative/antioxidative status assessed in the peripheral blood of patients suffering from different forms of urticaria. Thus, some changes in the metabolism of systemic ROS occur in patients with physical urticaria, but not in patients with CIU and NSAID-induced urticaria. However, the significance of these alterations in the pathogenesis of physical urticaria is unclear. The imbalance is likely to reflect alteration of cytokine production or activation of the complement pathway and could be a common biochemical basis for increased skin susceptibility to external stimuli in patients with cold and solar urticaria [7].

Taken together, indices of ROS activity, such as the activity of CuZn/SOD, GSH-Px, and CAT, as well as the MDA level in plasma and erythrocytes did not differ significantly between NSAID-induced urticaria patients and healthy controls. It seems that processes associated with NSAID-induced urticaria may not modify antioxidant enzyme activity and may not enhance lipid peroxidation in peripheral blood. The problem of oxidative stress should be further investigated and the study extended to include a larger number of patients suffering from different forms of this disease.

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