Nitric oxide and malondialdehyde levels in plasma and tissue of psoriasis patients

A. Şikar Aktürk,†* H.K. Özdoğan,‡ D. Bayramgürler,† M.B. Çekmen,‡ N. Bilen,† R. Kırarı̇n†
†Department of Dermatology, and ‡Department of Biochemistry, Kocaeli University Faculty of Medicine, Kocaeli, Turkey
*Correspondence: Dr. A. Şikar Aktürk. E-mail: aysun9442@hotmail.com

Abstract

Background The pathogenesis of psoriasis has not been known exactly yet. Recently, it has been suggested that increased reactive oxygen species (ROS) such as nitric oxide (NO) and malondialdehyde (MDA) may play a part in the pathogenesis of various skin diseases, including psoriasis.

Objectives In this study, we aimed to investigate the role of ROS in the pathogenesis of psoriasis.

Methods A convenience sample of 23 patients with psoriasis and 23 healthy subjects consented to participate in the study. Plasma NO and MDA levels were measured in all participants. Psoriasis area and severity index (PASI) and tissue levels of MDA on lesional and non-lesional skin regions of psoriasis patients were measured. In addition, the correlation between age, gender with plasma NO, plasma MDA and tissue MDA was assessed.

Results Plasma levels of NO and MDA in psoriasis patients (135.8 μmol/L, 4.33 μmol/L, respectively) were statistically significantly higher than those in controls (33.6 μmol/L, 2.03 μmol/L, respectively). Tissue levels of MDA in lesional tissues (49.18 nmol/gr) were significantly higher than those in non-lesional tissues (28.41 nmol/gr). A significant correlation was not found between the PASI and levels of NO and MDA. In addition, a significant negative correlation was found between the plasma NO levels and age.

Conclusion NO and MDA levels are elevated in psoriasis patients, which may indicate that oxidative stress plays an important role in the aetiopathogenesis of psoriasis.

Received: 25 January 2011; Accepted: 6 June 2011

Conflict of interest

None.

Funding support

None.

Introduction

Psoriasis is a common chronic skin disorder mediated by cellular immune mechanisms.1–4 Although it affects 1–4% of the general population worldwide,1,2 its pathogenesis is unknown.3 Epidermal keratinocyte activation, neutrophilic infiltration and increased synthesis of some cytokines are important events contributing to its pathogenesis, but the underlying cause and exact sequence of these processes are not known.2–4

Oxygen free radicals (OFRs), generally known as reactive oxygen species (ROS), along with reactive nitrogen species (RNS), play a dual role in biological systems; they may have harmful or beneficial effects.5–7 These include molecular oxygen (O2), superoxide anion (O2·−), hydrogen peroxide (H2O2), hydroxyl radical (OH), nitric oxide (NO), peroxynitrite (ONOO−) and hypochlorous acid (HOCl).4,5 OFRs are generated in the skin and other tissues exposed to ultraviolet light and O2. Skin is equipped with both enzymatic [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP)] and non-enzymatic mechanisms (vitamin C, vitamin E, carotenoids, thiol antioxidants, flavinoids, selenium and others) to deal with the harmful effects of ROS.5,6,8 Overproduction of OFRs results in oxidative stress leading to damage to cellular structures such as lipids, proteins and DNA,3,6 and interference with production of cytokines such as interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-α). Malondialdehyde (MDA) is an important product of lipid peroxidation; its levels correlate closely with the degree of lipid peroxidation in tissues.6,9,10 Researchers have recently suggested that OFRs play a central role in the induction of skin diseases such as psoriasis.1,11–17 atopic dermatitis, acne, irritant contact dermatitis, physical urticaria1 and rosacea.9
In this study, we aimed to investigate the role of OFRs in the pathogenesis of psoriasis by measuring plasma levels of NO, a ROS and MDA in psoriasis patients and controls, and tissue levels of MDA in affected and unaffected areas of skin in psoriasis patients.

Materials and methods

Patients
The study protocol was approved by the Ethics Committee of our university (approval 2007/5). A convenience sample of patients with active psoriasis presenting to the dermatology clinic of our tertiary care university medical centre was approached for inclusion in the study. Patients with additional dermatological disorders, any systemic disease or those who had received any systemic and topical treatment during the previous 4 weeks were not included in the study. Healthy volunteers were also recruited and served as controls. Volunteers and patients granting written informed consent participated in the study. History of patients was obtained and physical examination was performed in all participants and blood and tissue samples were obtained as described below. The psoriasis area and severity index (PASI) was calculated for the psoriasis patients.

Preparation of plasma and tissue samples
Ten mL of venous blood was taken from all subjects into additive-free vacutainer tubes and immediately transported to the laboratory. The blood was centrifuged at 1000 g for 10 min to separate the plasma. After plasma was removed, the remainder was stored at −80 °C for later measurement of NO and MDA levels. In addition, two punch biopsy samples were obtained from psoriasis patients from lesional and non-lesional (as control) skin regions on the trunk (from an area protected from sunlight). The biopsy samples were weighed, washed three times with 0.9% NaCl and then homogenized in four volumes of ice-cold buffer containing 20 mM Tris and 10 mM EDTA (pH 7.4). The washed and buffered tissue samples were immediately stored at −80 °C for later measurement of MDA. Measurement of NO in the tissue samples could not be performed for technical reasons.

Assay of plasma NO levels
Total nitrite (NO₂⁻) was quantified by the Griess reaction after incubation of supernatant with Escherichia coli nitrate reductase to convert NO₃⁻ to NO₂⁻.¹⁸,¹⁹ Griess reagent (1 mL 1% sulfanilamide, 0.1% naphthylethylenediamine hydrochloride, and 2.5% phosphoric acid) was then added to 1 mL of supernatant. Absorbance was read at 545 nm (UV mini-1240 spectrophotometer, Shimadzu Corp., Kyoto, Japan) after a 30-minute incubation. The absorbances were compared with a standard of NaNO₂, obtained from reduction of NaNO₃ (1–100 μmol/L). The reaction was linear from 0.25 to 100 μmol/L. The results were expressed as μmol/L in plasma.

Assay of plasma and tissue MDA levels
Thiobarbituric acid (TBA) reacts with lipoperoxidation aldehydes, such as MDA, as the most common method to assess lipid peroxidation in biological samples. The procedure was modified from Buege and Aust.²⁰ BHT was used to prevent lipid peroxidation during heating. Briefly, 0.5 mL of plasma or tissue homogenate was added to a reaction mixture (1.0 mL) formed by equal parts of 15% trichloroacetic acid, 0.25 N HCl and 0.375% TBA, plus 2.5 mM butylated hydroxytoluene (BHT) and 0.1 mL of 8.1% sodium dodecyl sulphate (SDS), followed by 30 min heating at 95 °C, the pH value of the analytical reaction mixture was about 0.9. After cooling, the chromogen was extracted with N-butanol and read spectrophotometrically at 532 nm (UV mini-1240 spectrophotometer, Shimadzu Corp., Kyoto, Japan) against a reaction mixture blank lacking plasma but subjected to the entire procedure and extracted with n-butanol. The results were expressed as μmol/L in plasma and nmol/gr in wet tissue according to a standard, which was prepared with serial dilutions of standard 1,1,3,3-tetramethoxyx propane.

Statistical evaluation
All data are expressed as mean and standard deviation (mean ± SD) and 95% confidence intervals. Pearson’s correlation coefficient was calculated to determine the correlation between PASI score and MDA and NO levels. Spearman’s rank correlation test was used to find the correlation between the age and plasma NO, plasma MDA and tissue MDA levels. Mann–Whitney U-test was used for comparisons between the gender and plasma NO, plasma MDA and tissue MDA levels. As the group variances were homogenous, data were compared between groups using parametric Student’s t test. P < 0.05 was considered statistically significant.

Results
Study participants included 23 psoriasis patients (52% female; mean age 42.8 ± 16.5 years, range 18–70 years) and 23 controls (52% female; mean age 42.2 ± 15.9 years, range 18–68 years). Of the 23 patients, five were diagnosed with guttate psoriasis, ten with generalized plaque-type psoriasis and eight with localized plaque-type psoriasis. Nail involvement of psoriasis was detected in 13 (56.6%) patients and psoriatic arthritis was found in three (13%) patients.

Plasma NO levels in psoriasis patients were significantly higher (mean ± SD: 135.8 ± 37.7 μmol/L) than those in controls (mean ± SD: 33.6 ± 20.3 μmol/L, P = 0.00). Plasma MDA levels in psoriasis patients (mean ± SD: 4.33 ± 1.20 μmol/L, Fig. 1) were significantly higher than those in controls (mean ± SD: 2.03 ± 1.28 μmol/L, P = 0.00). MDA levels were significantly higher in lesional tissues (mean ± SD: 49.18 ± 26.74 nmol/gr tissue, Fig. 2) than those in non-lesional tissues (mean ± SD: 28.41 ± 16.16 nmol/gr tissue, P = 0.003) (Table 1).

Psoriasis area and severity index scores ranged from 1.2 to 46.4 (mean 9.5 ± 9.9) (Fig. 3). PASI scores were not significantly correlated with plasma or tissue levels of NO and MDA in psoriasis.
patients \( r = 0.26, P = 0.54 \) for plasma NO and \( r = 0.14, P = 0.53 \) for plasma MDA and \( r = 0.14, P = 0.27 \) for tissue MDA).

Plasma NO, plasma MDA and tissue MDA levels were not significantly associated with gender (Table 2). But, plasma NO levels had a significantly negative correlation with age \( r = -0.425, P = 0.043 \). Plasma and tissue MDA levels were not significantly correlated with age \( r = -0.051, P = 0.817 \) and \( r = 0.099, P = 0.653 \), respectively).

**Discussion**

Skin is a major target of oxidative stress because of ROS originating from the environment and skin metabolism.\(^1,4,8-10\) NO and superoxide anions are oxidatively active molecules\(^5-7\) and MDA is the final product of the peroxidation process.\(^5,6,9,10\) In recent years, the role of ROS has been investigated in some dermatological diseases such as rosacea, vitiligo and photodermatoses.\(^4,9,10,14\)

Psoriasis is a common chronic skin disorder that is characterized by hyperproliferation, abnormal differentiation and altered maturation of the epidermis as well as inflammation in the epidermis and dermis.\(^7\) Although the exact cause remains unknown, environmental and genetic factors, as well as intracellular and intercellular mediators, are thought to play an important role in its pathogenesis.\(^1,2\) Some believe its pathogenesis is driven by activated T cells or antigen-presenting cells, chemokines and a number of inflammatory cytokines such as TNF-\( \alpha \), interferon gamma, interleukin-1.\(^1,2,21\) TNF-\( \alpha \) has been shown to cause high levels of inflammatory cytokines and a transcription factor in psoriatic patients.\(^1,10\) ROS have also been shown to be involved in TNF-\( \alpha \)-induced signalling pathways associated with certain inflammatory diseases such as psoriasis.\(^1,4\) In addition, cellular signal pathways such as mitogen-activated protein kinase/activator protein 1, nuclear factor \( \kappa B \) and janus kinase-signal transducers and activators of transcription are believed to be redox sensitive; these have been found to be involved in the progression of psoriasis.\(^1\)

Neutrophils, known as the best source of OFRs in organisms,\(^6,22\) are increased in psoriatic skin.\(^23\) For this reason, the role of OFRs in the aetipathogenesis of psoriasis has been investigated by numerous authors in recent years.\(^8,11-17\) Cals-Grierson and Ormerod suggested that NO stimulates the epithelial cells and releases chemokines and growth mediators which are important for the

**Table 1** The mean plasma levels of NO and MDA and the mean tissue levels of MDA in patient and control groups

<table>
<thead>
<tr>
<th></th>
<th>Patient group ((n = 23))</th>
<th>Control group ((n = 23))</th>
<th>(P)-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>The mean level of plasma NO (mean (\pm) SD) ((\mu\text{mol/L}))</td>
<td>135.8 (\pm) 37.7</td>
<td>33.6 (\pm) 20.3</td>
<td>0.00</td>
</tr>
<tr>
<td>The mean level of plasma MDA (mean (\pm) SD) ((\mu\text{mol/L}))</td>
<td>4.33 (\pm) 1.20</td>
<td>2.03 (\pm) 1.28</td>
<td>0.00</td>
</tr>
<tr>
<td>The mean level of tissue MDA (mean (\pm) SD) ((\text{nmol/g}))</td>
<td>49.18 (\pm) 26.74</td>
<td>28.41 (\pm) 16.16</td>
<td>0.003</td>
</tr>
</tbody>
</table>
pulmonary artery endothelial dysfunction. This might be because of the small number of the patient’s group. In addition, significantly negative correlation was found between plasma NO levels with age (r = -0.425, P = 0.043).

Drewa et al.\textsuperscript{13} examined erythrocyte concentrations of MDA and activity of CAT and SOD on particular days of treatment in blood samples of 67 patients with psoriasis and 35 healthy subjects. All patients were treated topically with salicyl ointment, cignoline and tar. Levels of MDA and activities of SOD and CAT were significantly different during treatment in the psoriasis patients compared with controls: MDA concentrations before treatment were 44% higher and SOD and CAT activities 20% and 27% lower than those observed in the controls. Dimon-Gadal et al.\textsuperscript{15} demonstrated increased oxidative stress in cultured psoriatic fibroblasts (as measured by $O_2^-$ and $H_2O_2$ levels) from both lesional and non-lesional skin. These results support the importance of oxidant-antioxidant processes in the aetiopathogenesis of psoriasis.

To date, no study in psoriasis patients has concurrently examined measures of oxidative stress (NO and MDA levels) in plasma, lesional tissue and non-lesional tissue. In only one trial, SOD and GP activity in erythrocytes and CAT activity and MDA levels in serum were compared in 22 psoriasis patients and 22 healthy controls; MDA levels in skin biopsies from both groups were also measured: significantly lower levels of erythrocyte SOD and GP activities, higher levels of serum CAT activity and tissue MDA levels were noted in the psoriasis patients, but no significant difference in serum MDA levels was found between the two groups. PASI scores were not reported.\textsuperscript{14} In our study, both tissue MDA levels in patients as well as plasma NO and MDA levels in patients and controls were measured. We considered the increased MDA levels in plasma and tissue to be associated with inflammation, but no correlation was found between PASI scores and MDA levels in our patient group. We thought that this finding might be because of the small number of the patient’s group.

The processes involved in the aetiopathogenesis of psoriasis are complex and often involve ROS. It is still unclear whether the observed abnormalities in ROS are causative of psoriasis or are only associated with the ongoing pathogenesis of psoriasis. Our data support the role of ROS and lipid peroxidation in the inflammatory response of psoriasis. Clarification of the role of oxidative stress in psoriasis may result in novel therapeutic approaches in the future.

**Acknowledgements**

The authors would like to thank Dr. Ayten Yazıcı and Dr. Melike Yavuz in Kocaeli University Faculty of Medicine for supporting this work.

**References**

Nitric oxide and malondialdehyde levels in psoriasis


