

INVESTIGATIVE REPORT

In vivo Assessment of Iron and Ascorbic Acid in Psoriatic DermisNATHALIE LEVEQUE¹, SOPHIE ROBIN², PATRICE MURET^{1,3}, SOPHIE MAC-MARY¹, SAFWAT MAKKI^{1,4}, ALAIN BERTHELOT², JEAN PIERRE KANTEIP³ and PHILIPPE HUMBERT^{1,*}*Laboratories of ¹Cutaneous Bioengineering, ²Physiology and Nutrition, ³Clinical Pharmacology and ⁴Galenic Pharmacy, Faculty of Medicine and Pharmacy, Besançon, France*

Reactive oxygen species play an important role in inflammatory skin diseases such as psoriasis. Reactive oxygen species synthesis is catalysed by iron and some species are scavenged by ascorbic acid. The aim of this work was to assess iron and ascorbic acid in uninvolved and involved psoriatic dermis and to compare the corresponding concentrations in the dermis of healthy subjects. Microdialysis associated with atomic absorption spectrometry and gas chromatography-mass spectrometry was used to assess iron and ascorbic acid, respectively. Seven psoriatic patients and five healthy volunteers were studied. Iron concentrations in the involved ($57.1 \pm 19.3 \mu\text{g/l}$) and uninvolved ($49.7 \pm 27.1 \mu\text{g/l}$) psoriatic dermis were higher than the corresponding value determined in the dermis of healthy subjects ($21.8 \pm 2.4 \mu\text{g/l}$) ($p < 0.05$). Ascorbic acid in involved ($47.3 \pm 8.2 \mu\text{g/ml}$) and uninvolved ($42.0 \pm 14.0 \mu\text{g/ml}$) psoriatic dermis was statistically lower than that found in healthy dermis ($176.8 \pm 29.0 \mu\text{g/ml}$) ($p < 0.05$). These results demonstrate that psoriatic patients exhibit high iron and low ascorbic acid concentrations in the dermis, but there were no significant differences between involved and uninvolved skin. **Key words:** atomic absorption spectrometry; gas chromatography-mass spectrometry; microdialysis.

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Psoriasis is a cutaneous inflammatory disease in which reactive oxygen species (ROS) are involved, albeit these are difficult to detect because of their high reactivity and extremely short half-life. Since ROS synthesis is catalysed by iron and some species are scavenged by ascorbic acid, indirect methods were developed as a measure by which ROS levels could be used to determine iron and ascorbic acid (1).

Iron and ROS are closely related (2, 3). In healthy conditions, most iron in the human body is bound to proteins (4). However, during the inflammation process, free iron, called 'catalytic iron', is released from storage

proteins such as ferritin (2, 5). There is a relationship between ROS formation and iron – ROS can release iron from protein storage and, vice versa, iron promotes the ROS formation responsible for frequent oxidative damage.

Skin possesses endogenous and exogenous antioxidant protectors to prevent oxidative damage. Among these skin antioxidants, ascorbic acid has demonstrated a protective role against UV injury (6). Therefore, both iron (pro-oxidant) and ascorbic acid (ROS scavenger) levels are of interest in psoriasis.

Contradictory results were found in the literature concerning iron in psoriatic skin (1, 7). K  k  am & Naziroglu (8) have indicated that psoriatic disease could be related to deficient functioning of the antioxidative system. In addition, in epidemiological studies it has been suggested that a diet rich in antioxidants, such as ascorbic acid, could decrease the risk of developing psoriasis (9).

Until now, *in vivo* determinations of iron and ascorbic acid in psoriatic dermis have been carried out using invasive methods such as biopsy (7, 10). In previous investigations on healthy subjects, a microdialysis method was used *ex vivo* to sample ascorbic acid (11) and iron (12) from human dermis. The microdialysis technique causes minimal tissue damage and no physical alteration to the skin (13).

The aim of this work was to assess iron and ascorbic acid levels in the involved and uninvolved psoriatic dermis versus healthy control dermis.

MATERIALS AND METHODS*Chemical products*

Iron (solution stock standard, 1 g/l) and HNO_3 (99%) were purchased from Prolabo (Paris, France). Ascorbic acid, para-hydroxybenzoic acid (internal standard) and *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA), a silylating agent, were purchased from Sigma Aldrich (St. Quentin Fallavier, France). Sterile Ringer solution (pH = 6, Cl^- : 111 mmol/l; K^+ : 5.3 mmol/l; Ca^{2+} : 1.8 mmol/l; Na^+ : 130 mmol/l, lactate: 27.6 mmol/l) was obtained from Maco Pharma (Paris, France).

Patients

Seven men with psoriasis were enrolled in the current study (46.1 ± 14.9 years, mean $\pm$ SD). The patients were not

receiving any medication. Following the collection of iron and ascorbic acid by microdialysis, they were treated for the first time with PUVA therapy. Microdialysis probes were inserted into the psoriatic dermis area and into the uninvolved dermis of the same subjects. Five healthy men were recruited as controls (39.4 ± 10.2 years old). Microdialysis probes were inserted into the flexor forearm dermis of healthy and psoriatic subjects. None of the patients had cutaneous cancer, hepatic disorders or renal disease. The study was approved by the local ethics committee. Informed consent was obtained from all subjects.

Iron and ascorbic acid analysis by microdialysis

The microdialysis system consisted of a CMA/100[®] syringe pump (Phymep, Paris, France) that delivered Ringer lactate solution to a CMA/140[®] microfraction collector. The microdialysis probes (CMA/20[®]) have a 20 kDa cut-off with a polycarbonate membrane (length 10 mm). The probes were sterilized using ethylene oxide. Sampling was performed by placing a tubular microdialysis membrane in the dermis parallel to the skin surface. The dialysis membrane is continuously perfused with a physiological fluid, which creates a concentration gradient along the probe. Compounds diffuse through the membrane from the interstitial fluid to the perfusate or from the perfusate to the interstitial fluid (14). The outlet perfusates, which contain ascorbic acid and iron, are collected in microtubes and assessed by gas chromatography-mass spectrometry and atomic absorption spectrometry.

After cutaneous application of an anaesthesia cream (Emla[®], Astra, France), probes were inserted into the patient dermis. Probes were perfused with Ringer lactate solution (pH=6) at a flow rate of 3 µl/min. Iron and ascorbic acid dermis samples were collected by microdialysis every 30 min over a 5 h period.

Iron was analysed by atomic absorption spectrometry (12). Iron concentrations in microdialysates were assessed using our previously validated method (12). Briefly, samples were prepared by adding 450 µl of 1% HNO₃ solution to 150 µl of microdialysates; 40 µl of each preparation was injected into the atomic absorption spectrometry; 50 µl of serum was added to 550 µl of 1% HNO₃ solution; and 40 µl of each preparation was injected into the atomic absorption spectrometry.

Assessment of ascorbic acid in microdialysates was performed in accordance with our previously validated method (11), i.e. 50 µl of serum was placed in vials and 1 ml of trichloroacetic acid (10%) and 1 ml of diethyl ether were spiked to the plasma. The organic phase was removed and placed in a new tube containing 50 µl of internal standard. The mixture was frozen, lyophilized and derivatized for 1 h at 80°C with MTBSTFA. Four microlitres of this solution was injected into the GC-MS apparatus.

Statistical analysis

A non-parametric test was used. Significance of the differences was accepted when the *p*-value was less than 0.05.

RESULTS

Iron concentrations (mean $\pm$ SD) in the involved and uninvolved psoriatic dermis and in the dermis of healthy subjects were 57.1 ± 18.3 µg/l, 49.7 ± 27.1 µg/l ($n=7$) and 21.8 ± 2.4 µg/l, respectively (Tables I and II).

Table I. Iron and ascorbic acid concentrations in psoriatic dermis (7 male patients)

Patient/age (years)	Iron (µg/l)		Ascorbic acid (µg/ml)	
	Uninvolved	Involved	Uninvolved	Involved
1/25	43.6	60.6	58.2	64.5
2/30	99.6	79.2	36.0	25.8
3/44	23.6	81.2	49.2	33.2
4/62	73.2	54.7	47.1	38.6
5/64	37.6	32.6	47.9	33.1
6/45	41.2	40.4	37.6	57.5
7/53	28.8	50.8	55.1	41.6
Mean $\pm$ SD	49.7 ± 27.1	57.1 ± 18.3	47.3 ± 8.2	42.0 ± 14.0
Median	41.2	54.7	47.9	38.6

Table II. Iron and ascorbic acid concentrations in healthy dermis (5 male controls)

Subjects/age (years)	Iron (µg/l)	Ascorbic acid (µg/ml)
1/42	18.4	210
2/45	20.4	200
3/27	24.7	159
4/31	21.9	176
5/52	23.5	139
Mean $\pm$ SD	21.8 ± 2.4	176.8 ± 29.0
Median	21.9	176
Comparison with uninvolved and involved psoriasis skin (see Table I)*	Uninvolved $p=0.0312$ Involved $p=0.0029$	Uninvolved $p=0.022$ Involved $p=0.042$

*Non parametric test.

There were no statistical differences between iron concentrations in involved and uninvolved psoriatic dermis ($p>0.05$). However, compared to the dermis of healthy subjects there was a statistical difference between the concentrations of iron in both involved ($p<0.003$) and uninvolved psoriatic dermis ($p<0.05$).

Ascorbic acid concentrations in psoriatic lesions and non-lesions were 42.0 ± 14.0 µg/ml and 47.3 ± 8.2 µg/ml, respectively ($p=0.301$; ns) (Table I). On the other hand the concentration in the dermis of healthy subjects was 176.8 ± 29.0 µg/ml (Table II) which is statistically different from the values in both types of psoriatic dermis ($p<0.05$).

Iron and ascorbic acid serum concentrations of psoriatic patients were 0.61 ± 0.23 mg/l and 43.5 ± 0.5 µg/ml, respectively (for controls: 0.5–1.7 mg/l and 7–18 mg/l, respectively).

DISCUSSION

Microdialysis offers two major advantages compared to biopsies: microdialysis is a slightly invasive technique and, in addition, proteins are not collected by this

Table III. Comparison of different techniques used to assess iron concentrations in psoriatic skin

	Molin & Wester 1973 (1)	Kurz et al. 1987 (7)	Forslind et al. 1999 (18)	This study
No. of subjects	9 psoriatic patients 4 control subjects	5 psoriatic patients 4 control patients	5 psoriatic patients 8 control subjects	7 psoriatic patients 5 control subjects
Tissue	Epidermis	Epidermis	Epidermis	Dermis
Sampling method	Biopsy	Biopsy	Biopsy	Microdialysis
Inclusion of iron binding proteins in the assay	Yes	Yes	Yes	No
Normal skin	26.5 ± 1.7 µg/g	240 ± 225 µg/g	105 ± 10.6 µg/g	21.8 ± 1.2 µg/l
Uninvolved psoriasis	30.6 ± 2.5 µg/g	98 ± 54 µg/g	141 ± 14.5 µg/g	49.7 ± 27.1 µg/l
Involved psoriasis	57.5 ± 2.9 µg/g	55 ± 23 µg/g	–	57.1 ± 18.3 µg/l

method. Consequently, no extraction is necessary prior to sample analysis. Microdialysis collects free iron (ferrous and ferric ions) as well as low molecular weight iron compounds (15, 16). Free iron is responsible for the ROS formation involved in inflammatory cutaneous diseases such as psoriasis (2). Determination of cutaneous iron concentrations might therefore reveal the relationship between iron and inflammatory processes.

Iron serum concentrations were normal in psoriatic patients (0.61 ± 0.23 mg/l) and in healthy subjects ($0.5\text{--}1.7$ mg/l) (17), whereas iron concentrations were high in psoriatic involved and uninvolved dermis compared to healthy dermis (Tables I and II).

Conflicting data have been published concerning iron concentrations in psoriatic skin. Molin & Wester (1), Naldi et al. (9) and Forslind et al. (18) found increased iron concentrations in psoriatic epidermis compared to control samples. Kurz et al. (7), on the other hand, reported decreased iron levels in epidermis of chronic psoriatic plaques compared to healthy control areas and Dogan et al. (19) stated that iron concentrations in the skin of psoriatic patients decreased due to an increased iron mobilization. Sato (20) indicated that iron concentrations in inflammatory skin diseases such as psoriasis might be higher than those in the controls. Our data concerning the iron in dermis tissues were in correlation with Sato's hypothesis.

In our *in vivo* study, iron concentration in normal dermis (21.8 ± 2.4 µg/l; Table II) was higher than in a previous *ex vivo* investigation (5.8 ± 2.0 µg/l) (12) using microdialysis. This difference could be explained by the fact that blood flow *in vivo* could affect iron recovery and consequently increase its basal concentration (21).

Owing to the lack of a non-invasive technique, previous determinations of antioxidant concentrations have been carried out using blood from psoriatic patients (2, 22). According to Trenam et al. (2) and Severin et al. (22), the concentrations of beta-carotene, tocopherol and urate in plasma are significantly higher in psoriatic patients compared to controls. Severin et al. (22) also indicated that vitamin E and ascorbic acid levels in plasma did not differ statistically from those in the controls. Our results for plasma ascorbic acid are in

a good agreement with this (43.5 ± 0.5 µg/ml; normal range 6–20 µg/ml (23)). Ascorbic acid concentrations in psoriatic dermis, however, were statistically lower than the control levels (Table I).

No statistical difference of iron or ascorbic acid concentrations was detected between involved and uninvolved psoriatic dermis of the same patient. Our results were in good accordance with those of Dimon-Gadal et al. (23), who demonstrated that there was no difference in oxidative damage to cultured fibroblasts prepared from skin with and without psoriatic lesions of the same patient. In addition, according to these authors, more oxidative damage occurred in psoriatic fibroblasts compared to control healthy fibroblasts.

In healthy subjects, most iron in the body is protein bound. During inflammation, free iron ('catalytic iron'), may be released from storage proteins (24), and concomitantly, ferritin synthesis increases (25). Free iron is sequestered by ferritin. However, if the ferritin synthesis is not sufficient to deactivate all the free iron, the excess of free iron produces ROS, which is responsible for the inflammation. The high free iron concentration in psoriatic dermis found by us confirms that during this inflammatory process free iron is released from the storage proteins and then produces ROS, which are subsequently scavenged by antioxidants such as ascorbic acid. This may explain the low ascorbic acid dermis concentrations in psoriatic dermis.

Further investigations are currently in progress in our laboratory examining the relationships between other dermatological diseases and the dermal concentrations of iron and ascorbic acid using the microdialysis technique.

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