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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Topical CHM extract improves epidermal permeability barrier function in normal mouse skin.

Figure S2. Topical CHM extract increases epidermal lipid in murine model.

Table S1. Primer sequences.

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Letter to the Editor

Increased cysteinyldopa plasma levels hint to melanocyte as stress sensor in psoriasis

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Abstract: The possible role of melanocyte as a modulator of the inflammation and keratinocyte hyperproliferation in psoriasis has been hypothesised but never demonstrated on experimental basis. Aim of the present study was to assess whether plasma levels of 5-*S*-cysteinyldopa (CD), a metabolite reflecting melanocyte activity, undergo changes in association with psoriasis together with those of typical lipid peroxidation markers thiobarbituric acid reactive substances (TBARS). A group of 16 patients with psoriasis at different stage as indicated by the psoriasis area and severity index (PASI) were enrolled against an age and sex matched control group. Both TBARS ($P < 0.05$) and CD ($P < 0.005$) levels were higher than controls with statistical significance. After 1 month therapy the levels of either biomarkers decreased with respect to the starting values although with marked individual differences. CD may represent a novel and sensitive biomarker for the follow up of psoriasis and evaluation of the efficacy of therapeutic regimens beyond PASI determination.

Key words: Cysteinyldopa – lipid peroxidation markers – melanocyte – plasma – psoriasis

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Background

The association of oxidative stress with psoriasis is amply documented (1,2). An overproduction of reactive oxygen species overwhelms the antioxidant capacity resulting in oxidative damage of lipids and other cellular constituents. Increased levels of lipid peroxidation markers such as malondialdehyde (3) and oxidized low density lipoprotein (4) in the tissues, elevation of the thiobarbituric reactive substances (TBARS) (5) in blood, and accumulation of fatty acid hydroperoxides in skin scales (6) have been observed in psoriatic patients.

Recently a key role of melanocyte in psoriasis has been proposed (7): signals from central nervous system and directed to melanocyte may affect also basal keratinocytes stimulating an abnormal differentiation and hyperproliferation.

That melanocyte may respond to different stimuli from the environment is shown by the immediate and delayed pigmentation developed following solar radiation (8) and other injuries including wound healing (9). In addition to pigment production within melanosomes and their delivery to keratinocytes, melanocyte is able to secrete a wide range of signalling molecules like cytokines, neuropeptides and neurotransmitters, which are directed towards specific receptors in keratinocytes, lymphocytes, fibroblasts and mast cells (10).

All melanocytes with functioning tyrosinase produce 5-*S*-cysteinyldopa (CD) whose levels in serum and urine are related to the size and pigment forming activity of the melanocyte population (11). On the other hand CD levels in melanocytes, epidermis and blood seem not to depend on skin type (12,13). An increase of

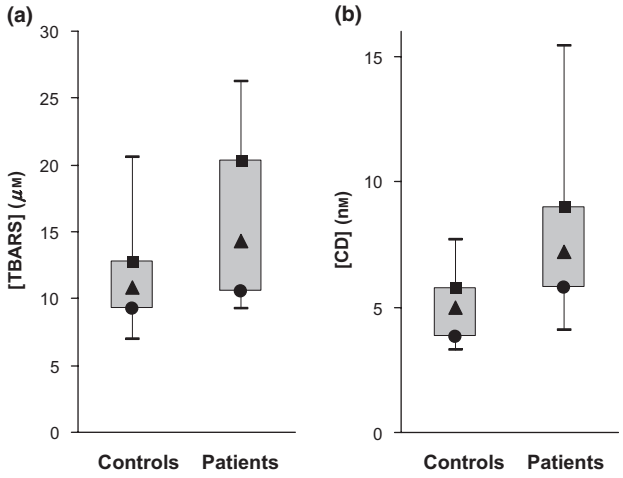

Figure 1. (a) TBARS and (b) CD plasma levels of psoriatic patients (n = 16) compared to controls (n = 15) (a, $P < 0.05$; b, $P < 0.005$). ●: 25th percentile; ▲: median;: ■: 75th percentile; –: minimum and maximum values.

CD excretion in body fluids is associated with exposure to UV-B (14) or UV-A (15) light radiation or phototherapy (16) as well as with pathological states, primarily melanoma, making this metabolite a valuable disease progression marker (17), but has more recently been observed in diseases not involving melanocytes, like severe renal failure with hemodialysis treatments, where the diffuse hyperpigmentation was shown to be due to accumulation of pheomelanin in the skin (18,19).

Questions addressed

The study was aimed at assessing changes of CD levels in association with psoriasis. Plasma levels of CD and TBARS were determined comparatively in a group of 16 patients with psoriasis at different stage as indicated by the psoriasis area and severity index (PASI) and an age and sex matched control group. In a restricted number of patients variations of such markers following therapy could be determined.

Experimental design

Patients

The present study was approved by the local ethics committee. All the patients and controls gave informed written consent.

Overall, 16 patients with psoriasis (14 men, 2 women; mean age 54.1 ± 14.8 years, range 25–79) and 15 healthy subjects (4 men, 11 women, mean age 44.0 ± 17.9 years, range 20–76) were enrolled in the study. Psoriasis was graded according to PASI exhibited at the time of blood collection (mean PASI 13.3 ± 5.2, range 7.3–26.1). Subjects were admitted only in winter and early spring, none of them had received UV radiation or any other specific therapy for at least 1 month prior to blood collection. Both patients and controls were not receiving any kind of medication, namely antioxidants, vitamins, or anti-inflammatories. Blood samples were drawn by venepuncture into vacuum tubes in the presence of heparin. Plasma was collected by centrifugation and stored at –80°C.

Analyses of CD and TBARS plasma levels

CD was prepared as reported (20). Determination of CD levels was performed using high-performance liquid chromatography as previously reported (21). Malondialdeyde and related lipid peroxidation products were determined using the spectrophotometric

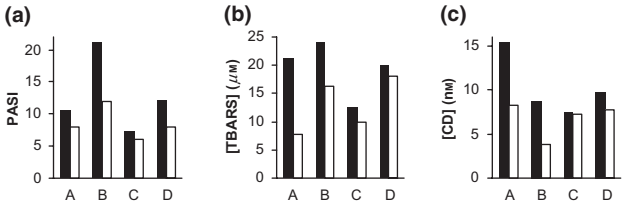

Figure 2. (a) PASI, (b) TBARS and (c) CD plasma levels of four psoriatic patients A–D before (black bars) and after (open bars) 1 month therapy.

TBARS assay according to protocols developed for plasma samples (22). TBARS were quantified by measuring the absorbance at 532 nm. The results were expressed as μmol/l according to a standard curve prepared using 1,1,3,3-tetramethoxypropane.

Statistical significance was evaluated using Student's test.

Results

The extent of lipid peroxidation in plasma was determined by measuring the levels of TBARS following current methodologies (22). Psoriatic patients exhibited a mean value of TBARS level of 15.6 ± 5.6 μM, significantly ($P < 0.05$) different with respect to the controls (mean value of 11.6 ±4.0 μM) (Fig. 1a).

Analysis of CD was performed using high performance liquid chromatography with electrochemical detection according to a well established procedure (21). Mean value of CD in psoriatic patients was 7.8 ± 3.2 nM, higher than that of controls of 4.9 ± 1.3 nM with statistical significance ($P < 0.005$) (Fig. 1b). No correlation between PASI and either TBARS or CD levels was observed.

In a few cases it was possible to follow patients during therapy. Remission of the disease associated with systemic or topical treatment was assessed by PASI determination (Fig. 2a). After 1 month treatment both TBARS and CD levels decreased with respect to the starting values although with marked individual differences (Fig. 2b,c). In two cases values at 2–4 months could be obtained but no significant further changes of the levels were observed (data not shown).

Conclusions

The positive association of psoriasis with levels of lipid peroxidation products like malondialdehyde and related TBARS has been extensively reported but data are often contradictory and scattered (5,23). Our data clearly indicate that psoriatic patients show an increased plasma levels of TBARS with respect to controls providing further support to the association of psoriatic condition exacerbation with chronic oxidative stress state. In addition, the observation that the levels of CD rise in association with psoriatic episodes is well in line with the reported ability of arachidonic acid metabolites resulting from phospholipases expressed in keratinocytes to stimulate melanocyte via specific receptors and rise tyrosinase activity responsible ultimately for CD production (24). Other inflammatory mediators and metabolites including notably histamine may as well activate tyrosinase via the cAMP pathway (25). On this basis the increase of CD levels in blood of psoriatic patients is likely to result from melanocyte stimulation, with tyrosinase mediated production of dopaquinone from tyrosine and its scavenging by cysteine. Yet, the possibility that CD may derive from oxidative conjugation of dopa and cysteine mediated by reactive oxygen species generated in an oxidative stress environment other than melanocyte as described *in vitro* (26,27) may not be definitely ruled out.

All together our findings would enforce the view that melanocyte may act as a sensory and regulatory cell of skin homeostasis as previously suggested (28,29).

Although further studies are needed to assess possible changes of CD levels in other non-pigmentary skin disorders, the present evidence points to CD together with oxidative stress markers as a promising diagnostic tool for the follow up of psoriasis and evaluation of the efficacy of therapeutic regimens including topical antioxidant/anti-inflammatory treatments (30). Development of easy to perform analytical protocols would therefore represent a focus of future research.

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Letter to the Editor

Decreased incidence of papillomas in mice with impaired EGFR function during multi-stage skin carcinogenesis

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Abstract: Genetically modified mouse lines revealed that the epidermal growth factor receptor (EGFR) is essential for the development and homoeostasis of the epidermis and hair follicles. However, more detailed studies have been precluded by the shortened lifespan of *Egfr* knockout mice. We employed the mouse line Wa5 (carrying a point mutation resulting in the expression of a dominant negative receptor) to analyse the impact of significantly reduced EGFR signalling during multi-stage chemical skin carcinogenesis. Seven-week-old Wa5 females and control littermates received a single application of 7,12-dimethylbenz(a)anthracene followed by multiple applications of 12-O-tetradecanoylphorbol-13-acetate for 26 weeks. Wa5 mice remained free of papillomas for a longer time and developed significantly fewer tumors than control littermates. In contrast, the mean tumor size was not different between groups. The present data indicate that EGFR signalling contributes to tumor growth during multi-stage chemical carcinogenesis of the skin in mice possibly by acting as a survival factor for skin tumor cells.

Key words: carcinogenesis – epidermal growth factor receptor – mutant mice

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Background

Activation of the epidermal growth factor receptor (EGFR) is a central event for normal skin development and homoeostasis and its deregulation can result in a variety of pathological conditions (1,2). EGFR signalling was also shown to support skin tumor development. For instance, targeted overexpression of the EGFR ligand transforming growth factor-α in the skin of transgenic mice induces epidermal thickening and the development of papillomas, preferen-

