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LETTER TO THE EDITOR

Nitric oxide levels in plasma and fibroblast cultures of psoriasis vulgaris patients

KEYWORDS
Nitric oxide; Psoriasis vulgaris; Fibroblasts; Human mast cell line (HMC-1)

Nitric oxide (NO) is an active molecule generated in many cells including fibroblasts and endothelial cells, participating in psoriatic inflammatory processes. In context of dermal vascular dilatation and increased blood flow, being characteristic features of psoriasis, the contribution of NO deserves special attention, however literature data on NO production in psoriatic plaques are inconsistent [1—3].

After Foreman's observations on inflammation development after neuropeptide release, numerous authors pointed out at the importance of neurogenic inflammation in psoriasis [4]. Moreover, literature data point out at mast cell involvement in psoriasis expressed by degranulation, increased histamine release and mast cell-nerves contacts [5]. Fibroblasts, regarded by many authors as only static elements of the extracellular matrix, fairly recently were revealed to actively participate in inflammatory and immune responses [6].

In view of the above data, the aim of the study was to evaluate NO levels in plasma of psoriatic patients and supernatants of psoriatic fibroblasts with human mast cell line (HMC-1).

Patients (76 males, 30 females, aged 19—79 years) with active psoriasis vulgaris were included in the study and put on classical treatment. Severity of the disease was assessed by PASI score and before treatment ranged from 7.2 to 29.8. The control group comprised 40 healthy individuals age- and sex-matched. Local bioethics committee approval was obtained.

Fibroblast cultures were established from 10 normal subjects and 10 psoriatic patients by punch biopsy from the newly formed psoriatic plaques, close surroundings and healthy donors. The tissue was processed as described in detail elsewhere [7]. Confluent fibroblast cultures of passage 3 or 4 were used in the study. Cells from the human mast cell line HMC-1 (Mayo Clinic, MMV-88-049) were employed. The cells were treated with either substance P (SP: 10^{-15}, 10^{-13}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8} M) or vasoactive intestinal peptide (VIP: 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) and supernatants for NO assessment were collected. Nitrite was measured using the Griess reaction [8].

Values of $P < 0.05$ were considered statistically significant.

After 3 weeks of treatment there was a significant decrease in PASI score ranging from 2.8 to 19.5 ($P < 0.001$). NO levels were significantly higher before treatment in plasma of psoriatic patients in comparison to after treatment and the control group ($P < 0.001$) (Table 1). After treatment, a positive correlation between NO plasma levels and PASI was observed ($P < 0.05$) and between erythema and NO plasma levels before and after treatment ($P < 0.05$) (Fig. 1).

In controls, SP- and VIP-stimulated cultures significantly increased NO levels were observed in both perilesional fibroblasts and co-cultures with HMC-1 in comparison to both control fibroblasts and co-cultures with HMC-1. Perilesional fibroblasts produced significantly increased NO levels in comparison to lesional ones, when stimulated with SP (10^{-15}, 10^{-9} and 10^{-8} M) and VIP (10^{-9}, 10^{-8}, 10^{-7} M). Furthermore, co-cultures of HMC-1 with perilesional fibroblasts produced significantly higher levels of NO than HMC-1, both in control conditions and when stimulated with SP (10^{-13}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8} M) and VIP (all employed concentrations) (Table 2).

Gokhale et al. [9] demonstrated significantly increased mean serum NO level in 36 patients presenting different types of psoriasis (chronic, erythrodermic, pustular) and found significantly higher NO levels in patients with active disease as compared to normal individuals. Örem et al [10] observed low NO serum levels during the inactive phase in psoriatic patients (mean PASI 1.7).

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Table 1 Nitric oxide levels (μM/L) in the examined groups

Statistical parameters	Control group	Psoriatic patients before treatment	Psoriatic patients after treatment
PASI			
$X \pm$ S.D.	–	16.7 ± 5.7**	9.3 ± 4.1**
Range	–	7.2–29.8	2.8–19.5
V	–	34.1	44.7
PASI reduction (%)			
$X \pm$ S.D.	–	44.7 ± 15.9	
Range	–	15.6–76.4	
V	–	35.6	
NO			
$X \pm$ S.D.	0.1 ± 0.06*	0.56 ± 0.29**/*	0.41 ± 0.16**/*
Range	0.05–0.36	0.21–1.73	0.14–0.81
V (%)	54.5%	52.7%	39.3%
NO reduction (%)			
$X \pm$ S.D.	–	16.0 ± 27.9	
Range	–	−43.0–82.3	
V	–	174.4	
Erythema score			
$X \pm$ S.D.	–	3.4 ± 0.7**	1.2 ± 0.9**
Range	–	2–4	0–3
V (%)	–	20.5	71.9

PASI: psoriasis area and severity index; NO: nitric oxide; *X*: mean, S.D.: standard deviation; *V*: variability (%) (control: $n = 40$, psoriatic patients $n = 106$, $P < 0.001$); *$P < 0.05$ between control group and patients group; **$P < 0.05$ between groups before and after treatment.

Our results demonstrating significantly increased NO levels in plasma of active psoriatic patients seem to be in agreement with literature data [9,10]. Decreasing but still significantly higher NO plasma levels after 3 weeks of effective treatment suggest that the balance in NO metabolism has not been restored yet. A positive correlation between both PASI and erythema and NO plasma levels suggest that NO plasma levels reflect anti-psoriatic therapy effectiveness. This statement seems to be further supported by Ormerod et al. [1] hypothesis regarding NO as an important element in lesional erythema maintenance. Those researchers concluded that NO production was the most objective method in psoriatic lesion remission evaluation when comparing with ultrasound, reflectance colorimetry or computerized video analysis.

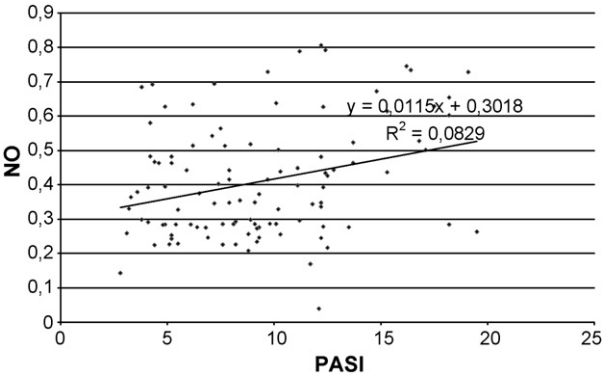

Fig. 1 Correlation between nitric oxide plasma levels (μM/L) and PASI after treatment. NO: nitric oxide and PASI: psoriasis area and severity index.

Human dermal fibroblasts spontaneously produce NO, however its significance in human dermal fibroblasts is not clear. Dimon-Gadal et al [2] demonstrated increased oxidative stress in psoriatic fibroblasts in lesional and lesion-free skin. Indeed, it has been suggested that fibroblasts may form a key element of an early warning system in tissues by producing chemokines in response to foreign intrusion [6]. Dimon-Gadal et al. [2] reported a positive correlation between the level of carbonylated proteins in psoriatic fibroblasts and IL-1β production in fibroblasts from uninvolved and involved psoriatic skin pointing out at non-lesional fibroblasts being already involved in psoriatic pathology, even before lesion formation.

To the best of our knowledge, there is lack of literature data on NO production by psoriatic skin fibroblasts and HMC-1 line co-cultures. Based on our results, it could be speculated that significantly higher level of NO production by perilesional psoriatic fibroblasts could facilitate expansion of psoriatic plaques. The above results seem to support