

## Short communication

# Increased serum nitrate levels in infants with atopic dermatitis

**Background:** The pathogenesis of atopic dermatitis (AD) is still unknown. A recent study has shown that inducible nitric oxide synthase (iNOS) is expressed in the atopic skin lesion, suggesting the involvement of nitric oxide in the skin inflammation of AD. The purpose of the study was to examine serum nitrate ( $\text{NO}_3$ ) levels in relation to the disease severity in children with AD.

**Methods:** Serum nitrate levels were assessed in relation to the skin scores in 88 patients with atopic dermatitis (AD) (aged 0.4–8 years: mean  $\pm$  SD,  $2.2 \pm 1.9$ , 41 boys and 47 girls) and 12 nonatopic children (aged 0.8–4 years: mean  $\pm$  SD,  $1.8 \pm 0.9$ , seven boys and five girls).

**Results:** Serum nitrate levels of patients with AD were significantly increased as compared to nonatopic controls and were also correlated with the disease severity. The skin scores were significantly correlated with serum nitrate levels as well as peripheral eosinophil counts.

**Conclusions:** Our results indicate that nitric oxide may be involved in the pathogenesis of vasodilation and erythema in AD skin.

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Nitric oxide (NO) has been found to be important in a number of different physiologic processes. Of particular relevance to the skin are the roles of NO in vasodilation, inflammation, immunomodulation, and oxidative damage to cells and tissues (1–3). There are three genetically distinct NO synthase (NOS) enzymes in man: two constitutive forms (neural NOS and endothelial NOS) and the inducible form (iNOS). Experiments with NOS inhibitors have indicated a role for NO in erythema and edema in psoriasis and atopic dermatitis (AD) (4). However, the direct measurement of NO is difficult due to its very short half-life in plasma (5).

NO reacts readily with molecular oxygen to yield nitrite and nitrate (6). NO, like nitrite, is oxidized very rapidly to nitrate by oxyhemoglobin (6). Fortunately, nitrate is stable and detectable in the serum (7).

Only a study about AD and plasma nitrate levels has been reported (8). In this study, we examined serum nitrate ( $\text{NO}_3$ ) levels in relation to the disease severity in children with AD, and we propose the hypothesis that nitric oxide is involved in the pathogenesis of vasodilation and erythema in AD skin.

## Material and methods

One hundred children were included in the study. The subjects were 88 patients with AD (aged 0.4–8 years: mean  $\pm$  SD,  $2.2 \pm 1.9$ , 41 boys and 47 girls) and 12 nonatopic children (aged 0.8–4 years: mean  $\pm$  SD,  $1.8 \pm 0.9$ , seven boys and five girls). The diagnosis was based on the criteria of Hanifin & Rajka (9). Informed parental consent was obtained.

Grading scores of AD were assessed by the scoring system proposed by Kimata & Igarashi (10). Briefly, grading of skin scores was 0–2 in the ascending order of severity with respect to inflammation, lichenification, and cracking, respectively. These signs were assessed on four areas of the body, i.e., face, trunk, arms, and legs, and the maximum possible score was 24. Scores for itching and sleep disturbance were graded 0–3 in the ascending order of severity, respectively. The total maximum possible score was therefore 30. The diagnosis of AD was classified as mild (grading scores: less than 10 points), moderate (11–20 points), and severe (21–30 points). None of the patients had received any topical and/or oral medications for at least 1 month before entering the study.

The control group consisted of patients with a negative personal history of atopy. Control children had no inflammatory skin disease.

A specimen of peripheral venous blood was collected to determine peripheral eosinophil counts and serum nitrate anion levels. All blood samples were drawn in the morning after fasting for 12 h except for infants. After centrifugation, the serum was stored immediately at  $-20^\circ\text{C}$  until analysis. Serum nitrate was measured by high-performance liquid chromatography with an anion exchange column (IC-PakA, Waters, Milford, MA, USA), as described by Takahashi et al. (7). Peripheral blood leukocytes were counted in a Coulter counter, and the eosinophil percentage was evaluated after May–Grünwald staining. Data were expressed as mean  $\pm$  SD.

Statistical analysis was performed with the nonparametric Mann–Whitney U-test for unpaired data and regression analysis with the Pearson correlation. A  $P$  value of  $<0.05$  was considered to be significant.

## Results

$\text{NO}_3$  levels of patients with AD were significantly increased as compared to nonatopic controls ( $41.5 \pm 29.6$  [ $n=88$ ] vs  $13.0 \pm 5.9$  [ $n=12$ ]) ( $P<0.001$ ), with  $115.5 \pm 46.2$  ( $n=4$ ),  $55.7 \pm 23.2$  ( $n=27$ ), and

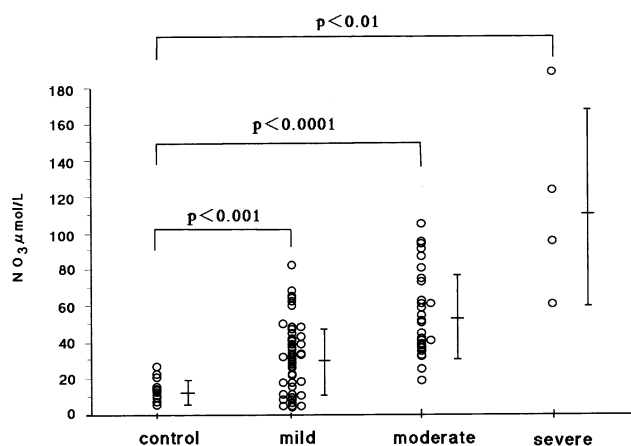


Figure 1. Serum NO<sub>3</sub> levels in mild, moderate, and severe AD patients, and nonatopic controls. Serum NO<sub>3</sub> levels in mild, moderate, and severe groups were significantly higher than nonatopic group ( $P < 0.001$ ,  $P < 0.0001$ ,  $P < 0.01$ , respectively).

$29.6 \pm 17.8$  ( $n = 57$ ) for severe, moderate, and mild AD, respectively (Fig. 1). There was a positive correlation between serum NO<sub>3</sub> levels and grades of skin scores ( $r = 0.592$ ,  $P < 0.0001$ ). There was also a positive correlation between eosinophil counts and serum NO<sub>3</sub> levels ( $r = 0.548$ ,  $P < 0.0001$ ). A weakly positive correlation existed between skin scores and eosinophil counts ( $r = 0.396$ ,  $P = 0.001$ ).

All patients received emollients and, if appropriate, topical steroids, topical tar, or antibiotics and antihistamine after entering the study. The scores, nitrate levels, and eosinophil counts were observed for an average period of 31 days (minimum to maximum, 17–45 days) in 12 infants. We compared these three parameters in 12 infants with AD before and after treatment. During therapy, serum nitrate levels significantly decreased in association with the recovery of skin conditions (Fig. 2), but no change of eosinophil counts was evident.

## Discussion

Nitrites and nitrates could be derived from extrinsic sources such as inspired air, ingested water, and food (11), and intrinsic sources such as activated macrophages, leukocytes (12), and endothelial cells (6). The extensive capillary networks including the endothelial cells of the body have a total surface of 700 m<sup>2</sup>, which is supposed to be large, and therefore most NO<sub>x</sub> (nitrites and nitrates) could be of endothelial origin (7). Therefore, vascular injury and/or stimulated endothelial cells may yield the endothelial nitrate (7, 13). iNOS immunoreactivity was found to be closely associated

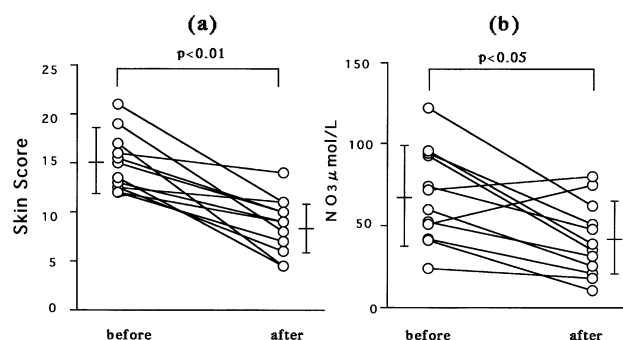


Figure 2. Changes in (a) serum NO<sub>3</sub> levels and (b) skin scores during treatment. Significant decreases of skin scores ( $P < 0.01$ ) and serum NO<sub>3</sub> levels ( $P = 0.012$ ) were found during treatment, but no change in eosinophil counts was evident.

with the upper dermal microvasculature in all inflammatory skin lesions of AD patients, but not in the noninflammatory area (14). The increased nitrate levels of AD patients' serum suggest that iNOS of endothelial cells in the atopic skin are stimulated and produce NO, which causes vasodilation associated with skin erythema or edema. Decreased nitrate levels during treatment may support this hypothesis.

iNOS is induced by various factors, among which the proinflammatory cytokines are the most thoroughly investigated (1, 2, 15). Specific cytokine combinations (IL-1 $\beta$ , IL-2 and TNF- $\alpha$ ) can induce iNOS *in vitro* (15). Although these cytokines are either absent or present only at low levels in AD lesions (16), other proinflammatory cytokines may be involved in the induction of iNOS in AD. iNOS is expressed by dermal endothelial cells or infiltrating cells closely associated with the endothelium; subsequently, the iNOS produces a relatively large amount of NO (17). This would stimulate vasodilation and subsequent erythema and edema, but could also be important in other aspects of the inflammatory and immune response, such as regulation of the expansion of the Th1 cell population (3). Thus, our results and previous studies have led to the hypothesis that raised levels of NO may interfere with the balance between Th1 and Th2 cell types (18).

Our results indicate that NO may be involved in the pathogenesis of vasodilation and erythema in AD skin.

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