

Influence of airborne nitrogen dioxide or formaldehyde on parameters of skin function and cellular activation in patients with atopic eczema and control subjects

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Indoor air quality is an important issue because residents of developed countries spend greater than 90% of their time indoors. Typical relevant pollutants of indoor air are tobacco smoke, CO, CO₂, NO, NO₂, radon, formaldehyde, volatile and semivolatile organic compounds, and asbestos. The impact of protein allergens from biologic material such as pollens, molds, or house dust mites on the manifestation of atopic diseases is well known.¹ However, as suggested by epidemiologic studies, low molecular air contaminants at common concentrations may also be of importance for human health. For example, studies with controlled exposure of patients with asthma to various nonbiologic pollutants showed that NO₂² or NO₂ plus sulphur dioxide³ enhance the airway response to inhaled allergens. NO₂ seems to be an aggravating factor for several atopic diseases.

Patients with atopic eczema have been only rarely assessed under defined conditions. We report here experiments in which we evaluated the effect of short-term exposure to NO₂ or formaldehyde on patients with atopic eczema or control subjects. To investigate the influence of NO₂ and formaldehyde on the epidermal barrier function and skin surface, transepidermal water loss (TEWL) and skin roughness were measured. Also, eosinophil cationic protein (ECP) and soluble interleukin-2 receptor (sIL-2R) levels in the serum were determined as markers of cellular activation, which correlate with the disease activity in atopic eczema.

Abbreviations used

ECP:	Eosinophil cationic protein
sIL-2R:	Soluble interleukin-2 receptor
TEWL:	Transepidermal water loss

METHODS

In this single-blind study, seven patients with atopic eczema, according to the definition of Hanifin and Rajka,⁴ or seven control subjects were exposed to NO₂ (patients with atopic eczema: 3 men, 4 women; median age, 27 years [range, 21 to 80 years]; control subjects: 3 men, 4 women; median age, 25 years [range, 20 to 33 years]), formaldehyde (patients with atopic eczema: 3 men, 4 women; median age, 24 years [range, 20 to 43 years]; control subjects: 4 men, 3 women; median age, 29 years [range, 22 to 33 years]), or room air (nonspecific exposure) (patients with atopic eczema: 2 men, 5 women; median age, 38 years [range, 24 to 73 years]; control subjects: 6 men, 1 woman; median age, 27 years [range, 22 to 32 years]) in the climate chamber for 4 hours (11 AM to 3 PM). Patients and control subjects were not identical in each exposure protocol. On the days the studies were performed, patients were not treated with ointments.

All exposures were conducted in a 60 m³ climate chamber in the Institut für Medizinische Balneologie und Klimatologie der Ludwig-Maximilians-Universität München. During the experiments, the temperature in the climate chamber was 22° C, and the air humidity was 30%. Room air without pollutants contained formaldehyde between 0.0044 ppm and 0.0054 ppm and NO₂ between 0.023 and 0.030 ppm. This climate chamber was used in a previous study.⁵

A shell with formalin solution (E. Merck, Darmstadt, Germany) was positioned in the air supplier of the climate chamber. The concentration was chosen according to the predetermined ventilation volume to obtain a concentration of 0.08 ± 0.016 ppm in the chamber. Aldehydes were measured as dinitrophenylhydrazine derivatives. Air sampling was accomplished with a volumetric pump (Desaga model GS 312, Heidelberg, Germany) with a sampling velocity of 1 L/min every half hour.

NO₂ calibration gas (1% NO₂ in nitrogen 5.0 [purity: 99.999%, vol]; Linde, Unterschleißheim, Germany) was introduced into the ventilation air by a flowmeter (Rotameter; Rota, Wehr, Germany) to obtain a concentration of 0.1 ± 0.02 ppm NO₂. The NO₂ concentration was determined continuously by means of a NO_x-monitor (NO_x-Analyser AC31m; Environment SA, Poissy, France). Median values of 15 minutes were registered.

TEWL was measured on noninflamed and nonscaling skin of both lower arms not covered by clothing with an evaporimeter (TEWAMETER TM 200; Courage and Khazaka electronic,

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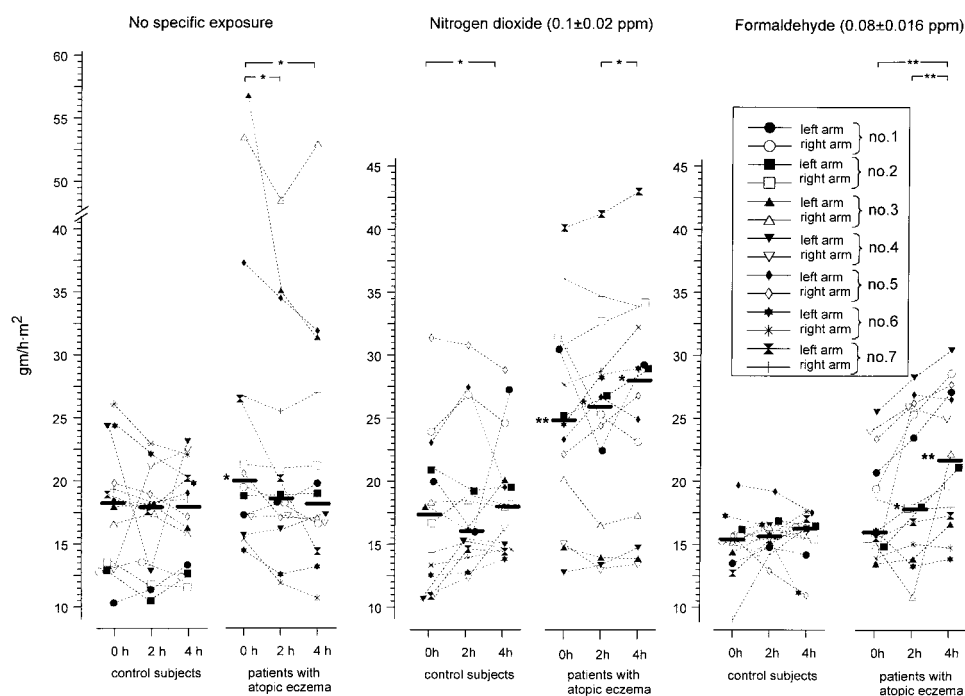


FIG. 1. TEWL of the same area of the left and right lower arm before (0 hours) and after 2 and 4 hours of exposure in patients with atopic eczema or control subjects. Individual values (two values of the lower arms of each person) characterized by different symbols within one exposure experiment are given. * $p < 0.05$; ** $p < 0.01$; * $p < 0.05$, median compared with control subjects; ** $p < 0.01$, median compared with control subjects.

Köln, Germany) before (0 hours) and after 2 and 4 hours of exposure. Values were displayed on a computer screen and the mean value during a period of 30 to 45 seconds after application of the probe on the skin was determined. To avoid influences of air movement, the test subjects placed their arms in a box in which measurements were performed.

Skin roughness was determined by taking replicas with silicone rubber dental impression material (Permadyne Garant, ESPE, Seefeld, Germany) of the surface of normal appearing skin of volar forearms not covered by clothing before (0 hours) and after 2 and 4 hours of exposure according to a previously described study.⁵

Blood was taken at 0, 4, and 24 hours for determination of ECP and sIL-2R levels in the serum. ECP was measured by a fluorescence enzyme immunoassay (CAP-System; Pharmacia, Uppsala, Sweden), and sIL-2R was measured by an ELISA (T Cell Diagnostics, Cambridge, Mass.).

The median and individual values of TEWL (characterized by different symbols for each test subject) are given. Statistical analysis was performed with the Wilcoxon test for paired comparisons and the Mann-Whitney U test for unpaired comparisons. Probability (p) values less than 0.05 were regarded as significant.

RESULTS

Control subjects and patients showed a significant increase of TEWL after exposure to NO_2 (control subjects: 0 hours, $17.45 \text{ gm/h} \cdot \text{m}^2$; 4 hours, $18.2 \text{ gm/h} \cdot \text{m}^2$; $p < 0.05$ and patients with atopic eczema: 2 hours, $26.2 \text{ gm/h} \cdot \text{m}^2$; 4 hours, $28.1 \text{ gm/h} \cdot \text{m}^2$; $p < 0.05$).

In patients with atopic eczema, TEWL was also significantly increased after exposure to formaldehyde (0 hours: $15.6 \text{ gm/h} \cdot \text{m}^2$, 4 hours: $21.6 \text{ gm/h} \cdot \text{m}^2$, $p < 0.01$; 2 hours: $17.8 \text{ gm/h} \cdot \text{m}^2$, 4 hours: $21.6 \text{ gm/h} \cdot \text{m}^2$, $p < 0.01$). In contrast, exposure to room air in the climate chamber reduced significantly the TEWL in patients with atopic eczema (0 hours: $20.2 \text{ gm/h} \cdot \text{m}^2$, 2 hours: $18.7 \text{ gm/h} \cdot \text{m}^2$, $p < 0.05$; 4 hours: $18.3 \text{ gm/h} \cdot \text{m}^2$; $p < 0.05$). At several points of measurement, patients with atopic eczema had significantly higher median TEWL values compared with control subjects (Fig. 1).

Skin roughness (arithmetic mean roughness value) was not influenced by exposure to room air in the climate chamber or by formaldehyde. Exposure to NO_2 caused a significant ($p < 0.05$) increase in skin roughness (median at 0 hours: $16.6 \mu\text{m}$; median at 4 hours: $17.2 \mu\text{m}$) in control subjects but not in patients with atopic eczema. There were no significant differences in skin roughness between control subjects and patients with atopic eczema (data not shown).

ECP levels in the serum were not influenced by the different exposures. Observed changes of sIL-2R serum levels seemed to follow a circadian rhythm and were unrelated to pollutant exposure (data not shown).

DISCUSSION

This study showed that short-term exposure to low concentrations of NO_2 or formaldehyde can induce skin

surface changes, especially a disturbance of the epidermal barrier function. For exposure with NO₂, a concentration of 0.1 ± 0.02 ppm was chosen. In homes with gas stoves, 0.025 to 0.075 ppm is a typical range of NO₂ concentration; peak values in kitchens with gas stoves or kerosene gas heaters range from 0.1 to 0.5 ppm. For formaldehyde exposure experiments, a concentration of 0.08 ± 0.016 ppm was used. In homes with urea formaldehyde foam insulation, formaldehyde concentrations were from 0.1 to 0.8 ppm; in homes without such insulation they ranged from 0.03 to 0.07 ppm.

Our results indicate that a short period of exposure to low concentrations of NO₂ affects the skin of patients with atopic eczema, as well as normal skin. It is known that NO₂ causes oxidative damage resulting in the generation of free radicals that may oxidize amino acids in tissue proteins. NO₂ also initiates lipid peroxidation of polyunsaturated fatty acids in pulmonary cell membranes. Similar mechanisms might be responsible for the effect of NO₂ on healthy skin, as well as for that on the skin of patients with atopic eczema. These changes evidently especially influence the epidermal barrier function as measured by TEWL, which is considered to reflect the functional state of the epidermal diffusion barrier and which increased significantly during NO₂ exposure. The finding of a decreased TEWL in patients with atopic eczema during their stay in the climate chamber without

specific exposure underlies the association between exposure to NO₂ and an impairment of the skin barrier.

Formaldehyde exposure induced a significant increase of TEWL in patients with atopic eczema but not in control subjects. The known irritant properties of formaldehyde may be the cause of this worsened epidermal barrier function.

The results of this study indicate that exposure to even low concentrations of pollutants for a short period of time can influence skin surface parameters, especially the skin barrier function. It might very well be that exposure to pollutants at higher concentrations, for a longer period of time, or both may lead to clinical deterioration of atopic eczema.

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