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Glyoxal-induced exacerbation of pruritus and dermatitis is associated with *staphylococcus aureus* colonization in the skin of a rat model of atopic dermatitis

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ABSTRACT

Background: Atopic dermatitis (AD) is a highly pruritic, chronic inflammatory skin disease associated with hyperreactivity to environmental triggers. Among those, outdoor air pollutants such as particulate matter (PM) have been reported to aggravate pre-existing AD. However, underlying mechanisms of air pollution-induced aggravation of AD have hardly been studied.
Objective: To investigate the molecular mechanisms by which glyoxal, a PM-forming organic compound, exacerbates the symptoms of AD induced by neonatal capsaicin treatment.
Methods: Naïve and AD rats had been exposed to either fresh air or vaporized glyoxal for 5 weeks (2 h/day and 5 days/week) since one week of age. Pruritus and dermatitis were measured every week. The skin and blood were collected and immunological traits such as Staphylococcus aureus skin colonization, production of antimicrobial peptides and immunoglobulin, and mRNA expression of inflammatory cytokines were analyzed.
Results: Exposure to glyoxal aggravated pruritus and dermatitis in AD rats, but did not induce any symptoms in naïve rats. Staphylococcus aureus skin colonization was increased in the skin of both naïve and AD rats. Expression of antimicrobial peptides such as LL-37 and β-defensin-2 was also increased by exposure to glyoxal in the skin of both naïve and AD rats. The mRNA expression of Th1-related cytokines was elevated on exposure to glyoxal. However, serum immunoglobulin production was not significantly changed by exposure to glyoxal.
Conclusion: In AD rats, exposure to glyoxal exacerbated pruritus and cutaneous inflammation, which was associated with increased colonization of *S. aureus* and subsequent immunological alterations in the skin.
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1. Introduction

Atopic dermatitis (AD) is a chronically relapsing inflammatory disease resulting from complex interactions between immunological and environmental factors [1]. Immunological features, including barrier dysfunction and type 2 T-helper cell (Th2) immune reactions, form the basis for initiation of AD pathology. Accordingly, acute lesions in the skin of patients with AD are characterized by profound increment of Th2 cytokines such as interleukin (IL)-4 and IL-5 [2], whereas in chronic AD lesions, type 1 T-helper cell (Th1) immune reaction coexists with Th2 immune reaction rather than a switch to Th1-dominant inflammation [3–5]. These intricate immune responses were possibly attributed to alteration of skin surface microflora [1,6]. *Staphylococcus aureus* has an ability to colonize the skin of patients with AD and is constantly found in eczematous skin lesions [7,8]. Importantly, the density of *S. aureus* colonization increased in the lesional and, to a lesser extent, in the non-lesional skin of patients with AD compared to healthy population. These changes were partially explained by the decrease in expression of antimicrobial peptides such as cathelicidins and defensins in patients with AD [9,10]. In addition, such increment in colonization was reported to not only correlate with the severity of diseases, but also contribute to the flare of AD [11–13].

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Moreover, the abnormal immunological traits of AD are poised to overreact to environmental stimuli such as air pollutants as well as allergens [14,15]. Among the environmental factors, air pollutants, including volatile organic compounds (VOC) and particulate matter (PM), have received increasing attention [16]. Epidemiological studies have found that VOC and PM not only have aggravating effects on patients with AD, but also contribute to the development of AD [16–19]. Toxicological studies have revealed the immunological characteristics associated with the aggravation of AD by environmental factors. For example, house dust mite allergen and formaldehyde increase the mRNA expression of tumor necrosis factor (TNF)-α and IL-1β in the skin of patients with AD and AD animal model, respectively [20,21]. On the other hand, benzene and its metabolites increase the number of IL-4-producing Th2 cells and reduce the number of interferon (IFN)-γ-producing Th1 cells. However, we still lack a detailed understanding of the underlying mechanisms by which PM exerts detrimental effects on manifestation of AD, since each environmental stimulus has been reported to exacerbate AD by different mechanisms.

In the present study, we investigated whether PM induces AD in naïve rats and/or exacerbates AD in an animal model of AD induced by neonatal capsaicin treatment [22–24], and also explored the underlying molecular mechanisms of the deteriorating effects of PM. For this purpose, vaporized glyoxal, a carbonyl compound that transforms into secondary organic aerosol as a major contributor to PM production [25,26], was used to investigate the effects of PM on AD and naive rats. First, we examined the symptoms of rats, and then evaluated the density of *S. aureus* colonization in the skin of AD rats and subsequent changes in the expression level of antimicrobial peptides. We analyzed the mRNA expression of Th1- and Th2-related inflammatory cytokines in the skin and the production of Th1- and Th2-related immunoglobulins in the serum.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Korea University College of Medicine Animal Research Policies Committee (KOREA-2016-0247). Pregnant Sprague-Dawley rats were acquired a week before parturition. After delivery, male pups were assigned randomly into four experimental groups. All animals were raised in a controlled environment at 22–25 °C and a 12 h light/dark cycle. All pups were allowed access to food and water ad libitum.

2.2. Neonatal capsaicin treatment

As described previously [27], newborn rat pups, within 48 h of birth, received subcutaneous injections of capsaicin (50 mg/kg; Sigma-Aldrich, USA) to induce AD in rats (AD rats). Only male rat pups were used for the experiment.

2.3. Glyoxal exposure

This procedure was previously described in detail [28]. Briefly, 40% glyoxal solution [29] (Sigma-Aldrich, USA) was vaporized using a Medical Regulator MB-10 (Chiyoda Seiki, Japan) with a flow rate of 8 mL/min. The vaporized gas was passed through a sealed chamber (50 cm × 35 cm × 25 cm) with two outlets for the input and output.

2.4. Establishment of animal groups

One week after birth, AD and naive rats were exposed to vaporized glyoxal solution or fresh air for 5 weeks (2 h/day and 5 days/week), thus establishing four groups: Gly-AD, Air-AD, Gly-naïve, and Air-naïve.

2.5. Scratching behavior

Rats were individually placed in plastic chambers (20 cm × 30 cm × 20 cm). A mirror was placed at the chamber's backside to avoid blind angle. Rat behavior was recorded with a digital video camera (DCR-SR300; Sony, Japan) in an isolated room. Watching the recorded video, the experimenter counted the number of scratches without knowing which group the rats belong to. Only the scratches using hind paw were accepted as valid, and a successive series of scratches were counted as one scratch.

2.6. Evaluation of cutaneous lesions

Cutaneous lesions were carefully examined and evaluated by scoring, as described previously [21]. The unit size for the extent of skin lesions was 0.25 cm^2, and the skin lesions were evaluated according to their severity, as shown in Table 1. The dermatitis score was calculated by the severity index multiplied by the unit size.

2.7. Collection of the serum and skin

After 6 weeks of behavioral tests, the rats were sacrificed using CO_2 gas and the blood in the right atrium was collected using a 1-mL syringe with a 26-gauge needle. The skin was obtained using a 2–4-mm punch each week, from the second week until the sixth week, for quantification of *S. aureus* colonization. For immunohistochemistry and qantitative real-time RT-PCR, the lesional skin in the middle of the back of the neck (or the nearest lesional part) was excised and acquired in AD rats. The non-lesional skin in the same part of the body was excised and acquired in naive rats. The samples were stored at −70 °C and used within 4 weeks of collection.

2.8. Quantification of *S. aureus* from the skin

The skin samples (20–50 mg) were homogenized using HG-15D (DAIHAN Scientific Co., Korea) at 6000 rpm for 10 s and diluted by a factor of 10 by serial dilutions. The diluted sample (100 μL) was spread on HiCrome Aureus Agar Base (Sigma-Aldrich, USA) plate. The plates were incubated at 37 °C for 24 h. The number of colonies was counted in duplicate and expressed as colony-forming unit (CFU) per mg of skin sample. Logarithm scale of the number of colonies was used for statistical analysis.

2.9. Immunohistochemistry (IHC)

The skin samples were fixed in 4% formaldehyde solution for 24 h and preserved in 0.01 M phosphate-buffered saline (PBS)

Table 1
Severity Index.

Face	0	Normal
	1	Wispy fur
	2	Alopecia or flare
	3	Bleeding or Scab
Ears	0	Normal
	1	Flare
	2	Bleeding or Scab
	3	Loss of the tissue
Back	0	Normal
	1	Wispy fur
	2	Alopecia or flare
	3	Bleeding or Scab

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