Perspiration promotes the effect of sulphite on the shielding response of rodent skin

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ABSTRACT

Perspiration and environmental chemicals, such as air pollutants, are two of the complicating factors of skin disease. It has not been studied how perspiration affect the skin responding to air pollutants. We applied topically artificial eccrine perspiration, sulphite or both to the mouse skin for one and two weeks to examine the influence of both factors on the shielding ability of healthy skin. Morphological examination showed apparent thickening of the epidermal layer in the skin samples with combined treatment at 1 week, and in the sections applied with sulphite and combined treatment at 2 weeks without significant difference in the extent of epidermal hyperplasia between two groups. The outcomes of immunohistochemical (IHC) analysis showed elevated percentages of dermal fibroblasts expressing interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), tumor necrosis factor β (TNF-β) and cyclooxygenase-2 (Cox-2). Results of two-way repeated measured analysis of variance (two-way RMANOVA) showed that both perspiration and sulphite, but not the interaction between them, were significant factors affecting the expression of proinflammatory cytokines. The evidences indicated that perspiration induced cytokines expressions in the dermal fibroblasts and promoted the effect of sulphite on the shielding response of the skin by inducing epidermis hyperplasia.

1. Introduction

Air pollution is an ongoing global health hazard. The precise composition of air pollutants varies widely depending on the local contributors within a geographical location and on the level and type of industrial activity involving thermal power plants, vehicular emissions, fossil fuel use, and agricultural activities. Therefore, the effects of air pollution on human health are often regionally specific. Studies have extensively investigated the contribution of air pollution to human diseases, such as those associated with the cardiopulmonary system and skin functions (Allen et al., 2008; Epton et al., 2008; Valacchi et al., 2012; Lademann et al., 2004; Vierkötter et al., 2010; Kim et al., 2013). Due to the differences between studies with respect to the experimental models (cellular, animal, or cohort study), air pollutants (individual components or mixtures), and human diseases in their analyses, the interpretation of the effects of air pollutants on human health achieved from different studies is sometime controversial. Sulphite, a derivative of sulphur dioxide in air pollution, was shown to intensify the toxic effects of inhalable particulate matters (PMs) in a cellular study that used simulators of particle-associated high-sulphur air pollution (Wang et al., 2019). Conversely, sulphur dioxide was not revealed to be associated with systemic lupus erythematosus in a cohort study investigating how long-term exposure to traffic-related air pollutants influences the disease (Jung et al., 2019).

Human skin provides the body with shielding protection against continual exposure to extrinsic chemical and physical air pollutants (Valacchi et al., 2012). Although studies have established the association between air pollution exposure and several skin conditions (Lademann et al., 2004; Vierkötter et al., 2010; Kim et al., 2013), climate is still an

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often overlooked factor that might influence the effect of air pollution on the skin. People living in tropical areas perspire more, and such perspiration accumulates on the skin. Water soluble air pollutants can dissolve in sweat (due to the high water content of perspiration) and subsequently adhere to the skin for a long time. Sweat is normally a transparent fluid that is 99% water and slightly acidic (pH 5–7) (Sat et al., 1989). In addition to water, sweat also contains small amounts of amino acids, urea, potassium, sodium, chloride ions, metabolites (such as lactate and pyruvate), and xenobiotics (Sat et al., 1989; Caplan and Goldberger, 2001; Raiszadeh et al., 2012). Studies have demonstrated that perspiration not only regulates body temperature but also provides protection to the skin because it contains dermcidin, which is a natural bactericidal peptide (Schittek et al., 2001). However, studies have proposed that unhealthy perspiration-related conditions influence many skin disorders, such as parapsoriasis and lichen amyloidosis (Hayakawa et al., 2013; Mizukawa et al., 1999), and exacerbate symptoms of atopic dermatitis and cholinergic urticaria (Hiragun et al., 2013).

Evidence from our previous study indicated that the skin, as part of its role as a barrier, may undergo increased epidermal hyperplasia when it is exposed to sulphite, and this protection is effective against a more complex and concentrated mixture of air pollutants (Chiu et al., 2020). Taiwan has a hot and humid climate and a large population of people riding motorcycles. Outdoor workers and riders tend to be exposed to air pollution daily under sweaty conditions. In the present animal study, sulphite was used as a representative to examine the influence of perspiration on the shielding ability of healthy skin when it responds to prolonged and repeated exposure to air pollution; ambient SO2 could absorb moisture or dissolve in perspiration to form sulphite.

2. Materials and methods

2.1. Antibodies and reagents

Artificial eccrine perspiration (pH 6.8) was obtained from Pickering Laboratories (USA). Primary antibodies used in immunocytochemical (IHC) analysis were as follows: anti-interleukin 6 (IL-6) (Abcam, Cambridge, MA, USA), anti-cyclooxygenase-2 (Cox-2) (Cell Signaling, Danvers, MA), anti-tumor necrosis factor α (TNF-α) (Novus Biologicals, Littleton, CO), anti-tumor necrosis factor β (TNF-β) (Affinity, Zhenjiang City, China), and anti-actin (Gene Tex Inc, San Antonio, TX).

2.2. Animal treatment

Male BALB/c mice, 5 weeks old, were obtained from the National Laboratory Animal Center (National Science Council, Taipei City, Taiwan). The use of mice was reviewed and approved by Chung Shan Medical University Animal Care Committee (IACUC approval number: 2080). The animals were housed in standard cages in a climate-controlled animal facility (temperature, 22 ± 2 °C; humidity, 55 ± 2%) with 12 h illumination daily at Chung Shan Medical University. After one week of adaptation, freely given standard rat chow (Purina Laboratory Chow obtained from Purina Mills, Inc., St. Louis, MO, USA) and drinking water, animals (n = 5) were shaved and the exposed dorsal skin was cleaned with ddH2O and divided into 4 parts (1 cm2 each) receiving topical application of the followings once daily for 1 and 2 weeks: 100 μL ddH2O, 100 μL H2SO3 (0.3 mM), 100 μL perspiration and 100 μL H2SO3 (0.3 mM) followed by 100 μL perspiration (Figure 1S for the scheme of treatments). The dosage of sulphite used in this study was relatively
equivalent to the one when atmospheric SO\textsubscript{2} was pumped and fully solubilized in 1 L of water for 24 h to mimic a prolonged and repetitive exposure of solubilized environmental SO\textsubscript{2} in our previous study (Chiu et al., 2020). At the end of the experiment, mice were euthanized by carbon-dioxide asphyxiation followed by exsanguination. The skin samples were collected and analysed by IHC.

2.3. Histological examination

Skin biopsies were fixed in 10% formalin immediately after removal from the animals, and processed by routine histology procedures for hematoxylin-eosin (H&E) staining. The skin sections (5 μm thick) were deparaffinized with xylene, hydrated in a descending series of graded ethanol, and stained with hematoxylin for 2 min, followed by rising in gentle running water for 2 min and eosin staining for 5 s. The histological change was examined by a pathologist. The extent of epidermal hyperplasia was quantitated manually by measuring the portion of hyperplasia relative to the full length of the skin section under the supervision of a pathologist and expressed as percentage.

2.4. IHC analysis

Skin sections (5 μm), formalin-fixed and paraffin-embedded, on coated slides were deparaffinized with xylene and rehydrated in a descending series of graded ethanol. The endogenous peroxidase activity was blocked with 0.6% H\textsubscript{2}O\textsubscript{2}. The sections were then incubated with primary antibodies at a dilution as suggested by the manufacturers at 37°C for one hour, followed by detection with UltraVision Quanto Detection System HRP and DAB Quanto Chromogen and Substrate (Thermo Scientific, USA), counter-stained with Mayer's hematoxylin and mounted in glycerin.

2.5. Statistical analysis

Results were presented as mean ± standard deviation (SD). Two-way repeated measured analysis of variance (two-way RMANOVA) was used to test a significant effect of perspiration, sulphite and interaction between two factors on the parameter (p values). Paired t-test was used to compare treatment groups with control. *p < 0.05, **p < 0.01, ***p < 0.001 vs control; ###p < 0.001 vs perspiration; ####p < 0.001 vs sulphite.

3. Results

3.1. Morphological change of skin induced by treatments

In order to minimize the individual differences among animals, the tested materials were topically applied to the same animal (n = 5) using ddH\textsubscript{2}O as control. Morphological examination of the H&E staining results of the skin sections showed that the thickness of epidermal layer was significantly changed by different treatments (Figure 1A, the representatives of skin sections from the same animals). At one week time point, the combined application of sulphite and perspiration increased the
width of the epidermal layer that was not affected when the reagents were used alone. The extent of epidermal hyperplasia that was quantitated by measuring the portion of hyperplasia relative to the full length of the skin section indicated that the combined treatment induced 50% more hyperplasia area than the other three groups (Figure 1B). As the exposure time was lengthened (2 weeks), perspiration remained unaffected to the morphological change, while sulphite had the same result as the combined group (Figure 1B). Two-way RMANOVA analysis of the data showed that sulphite alone, but not perspiration alone, was a significant factor of inducing epidermal hyperplasia, and there was no interaction between perspiration and sulphite affecting the response at 2 weeks. Besides the morphological change of epidermal layer, there was no other apparent alteration consistently observed among all animals.

3.2. Effect of perspiration and sulphite on expression of proinflammatory cytokine TNF-α, TNF-β and IL-6 in dermal fibroblasts

The induction of epidermal hyperplasia prompted us to look into whether there was an inflammatory response involved in the process. IHC analysis was performed to identify the expression of proinflammatory factor TNF-α, TNF-β, and IL-6.

3.2.1. IHC analysis of TNF-α

The IHC results showed that topical application of perspiration alone or combined with sulphite, but not sulphite alone, increased significantly the number of TNF-α positive fibroblasts in the dermis (Figure 2A arrows, and 2B) by a percentage of 25% or 130% of control value respectively at 1 week. Two weeks after the application, all the treatment groups had significant higher number of dermal fibroblasts expressing TNF-α than that of control by 64%, 48%, 141% for perspiration, sulphite and combined group respectively. The statistical analysis results of two-way RMANOVA showed that the percentage of dermal fibroblast expressing TNF-α was significantly influenced by perspiration and sulphite at both time points (P values in Figure 2B). Although no interaction between these two factors was observed, the percentage of TNF-α expressing fibroblasts was significantly higher in the combined samples than the other three groups at both time points (Figure 2B).

As for the analysis of TNF-β, the staining of antibody showed a significantly higher population of the dermal fibroblasts expressing TNF-β in the sulphite, alone or combined with perspiration, treated samples as compared to the control at 1 week by increasing a percentage of 117% and 158% for sulphite alone and combined treatment respectively (Figure 3A arrows, and 3B). When the treatment period was extended to 2 weeks, perspiration alone or combined with sulphite induced the expression of TNF-β. The combined treatment had more TNF-β-stained fibroblasts than the other three groups (Figure 3B). Sulphite expressed its influence at 1 week and perspiration at 2 weeks as analyzed by two-way RMANOVA (P values in Figure 3B). There was an interaction between these two factors affecting TNF-β expression at 2 weeks (P = 0.01). The combined treatment showed a higher population of TNF-β positive fibroblasts than the other three groups.
3.2.2. IHC analysis of IL-6

The antiserum-staining results revealed that there was an increase in the percentage of dermal fibroblast expressing IL-6 when the skin was treated topically with perspiration, sulphite or both at both time points (Figure 4A, arrows). The number of IL-6 positive fibroblasts was 2.2, 2.4 and 2.8 folds of control in the samples of perspiration, sulphite and combined treatment respectively at 1 week, and was even more significantly increased at 2 weeks (4.2, 4.6 and 6.0 folds) (Figure 4B). Perspiration and sulphite significantly influenced the expression of IL-6, but no interaction between them was observed at both time points (P values in Figure 4B).

3.2.3. Effect of perspiration and sulphite on expression of Cox-2 in dermal fibroblasts

Cox-2, an enzyme synthesizing prostaglandins, is an inducible early proinflammatory gene and is activated in response to various stimuli including TNFs (Medeiros et al., 2010). The IHC results showed that sulphite induced the expression of Cox-2 in the dermal fibroblasts at 1 week, and perspiration at 2 weeks (Figure 5A, arrows). The combined treatment had significantly more fibroblasts expressing Cox-2 than the other treatments at both 1 and 2 weeks (Figure 5B). Both perspiration and sulphite, but not the interaction between them, were significant factors affecting the expression of Cox-2 as shown by two-way RMANOVA analysis (P values in Figure 5B).

4. Discussion

Sweating is the process of releasing fluids to regulate body temperature. Perspiration replenishes moisture on the skin to maintain surface protection; however, skin moisture (which is influenced by relative humidity) is also a key determinant of human exposure that results in the increased dermal absorption of pesticides such as propoxur and chlorpyrifos (Williams et al., 2004; Meuling et al., 1997). Studies have delineated the association of skin conditions with air pollution and perspiration, but none have focused on how perspiration influences the response of the skin to air pollutant exposure. An understanding of the relationship between air pollution and perspiration in relation to healthy skin can elucidate the effect of these two factors on skin diseases.

In the present study, we determined the shielding response of the skin to sulphite when it is under the influence of perspiration. The combined application of sulphite and perspiration induced epidermal hyperplasia at 1 week to an extent comparable to that achieved at 2 weeks by sulphite and combined treatment, with perspiration continuing to have no effect on the skin. This observation indicated that perspiration was able to promote, but not enhance, the skin’s reaction to sulphite exposure. The results of an examination of the expressions of proinflammatory cytokines indicated a minor inflammation in response to sulphite and perspiration exposure. An additive effect was observed between these two factors; this was indicated by the fact that the percentage of dermal fibroblasts expressing the tested proteins (except for IL-6) was significantly higher in the combined treatment samples than in the single agent (either sulphite or perspiration) and control samples (Figures 2, 3, 4, and 5), particularly at 2 weeks. However, a two-way RMANOVA analysis indicated that perspiration did not interact with sulphite to influence the skin. The additive effect of perspiration and sulphite on cytokine expression by dermal fibroblasts was not reflected in the extent of epidermal hyperplasia; this indicated that the inflammatory stimulus of

Figure 4. Induced expression of IL-6 in dermal fibroblasts by perspiration, sulphite or combined treatment of both. (A) Representatives of histological images stained with IL-6 antiserum of dorsal skin tissues received topical application of perspiration, sulphite or combined treatment of both for 1 week and 2 weeks. (B) Percentages of IL-6 positive fibroblast. Scale bar, 100 μm. Arrow, IL-6 positive fibroblast. Results were presented as mean ± SD, and data were analyzed by two-way RMANOVA with a post-hoc analysis of Holm Sidak test to test a significant effect of perspiration, sulphite and interaction between two factors on the parameter (p values). Paired t-test was used to compare treatment groups with control. **p < 0.01 vs control.
perspiration could shorten the response time of the skin’s barrier function to a prolonged and direct exposure of sulphite, but did not increase the intensity of this response.

The dosage of sulphite used in this study resulted in the initiation of epidermal hyperplasia in 10 days; furthermore, the extent of the epidermal hyperplasia was comparable to the protection induced by the more complex and concentrated ambient air pollutants that we explored in our previous study (Chiu et al., 2020). Although epidermal hyperplasia is a key feature for diseases, such as atopic dermatitis and psoriasis, the pathological change of the skin is a manifestation caused by inflammatory stimuli originating from inside the body (Tamagawa-Mineoka and Katoh, 2020). Sweat and environmental chemicals, such as air pollutants, are two of the complicating factors of atopic dermatitis (Tamagawa-Mineoka and Katoh, 2020). The results of the present study revealed that both factors were able to induce the expression of proinflammatory cytokines in dermal fibroblasts; this likely contributes to the symptoms of dermatitis, which is an observation that has not been reported. The additive effect of perspiration and sulphite exposure is a key issue that must be considered when investigating the health hazards posed by air pollution to healthy and diseased skin.

The study of systemic sclerosis indicated that semaphorin 4A induced a profibrotic phenotype in dermal fibroblasts, which could play an essential role in inflammatory and fibrotic processes that influence the pathology of the disease (Carvalheiro et al., 2019). Activated dermal fibroblasts mediated skin fibrosis with IL-6 as a key mediator in systemic sclerosis (Denton et al., 2018). Additionally, a study revealed that the inflammatory dermal fibroblasts producing cytokines such as IL-6 promote the progression of psoriasis. Psoriasis is a common chronic inflammatory skin disease underlying the participation of dermal fibroblasts in the transition from innate to adaptive immunity (Angiolilli et al., 2021). Exposed to air pollution would provoke the psoriasis or make the lesion skin more sensitive to inflammatory cytokines from the microenvironment. Although perspiration was not observed to cause hyperplasia in healthy skin, it induced cytokine expression in dermal fibroblasts and promoted the effect of sulphite. This information is useful for developing prevention and care strategies for these diseases. We provided evidence verifying 1) that both perspiration and sulphite induced the expression of proinflammatory cytokines in dermal fibroblasts and 2) that perspiration promoted the epidermal hyperplasia induced by sulphite exposure. The additive effect of perspiration must be considered when investigating the health hazards posed by air pollution to skin, whether healthy or diseased.

In this study we raised the question of possible additive effect of perspiration on air pollution which is a complex mixture of thousands of components among which particulate matter, carbon monoxide, ozone, nitrogen dioxide and sulphur dioxide are considered major public health issues. Therefore, it is not appropriate to relate literally the results of present study to the action of air pollution. Further investigation will be needed to reveal the exact effect of perspiration.
 Declarations

Author contribution statement

Po-Ju Lai: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Fen-Pi Chou: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ting-Shuan Yu: Performed the experiments.

Huei-Jane Lee, Chun-Tung Chiu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

The authors do not have permission to share data.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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