Protective Effects of Topical Vitamin C Compound Mixtures against Ozone-Induced Damage in Human Skin

Journal of Investigative Dermatology (2017) **137,** 1373–1375; doi:10.1016/j.jid.2017.01.034

TO THE EDITOR

Environmental pollution is a challenge to modern society, especially in developing countries. It has been estimated that more than 90% of the urban population live with pollutant levels in excess of World Health Organization standard limits (World Health Organization, 2016).

There are numerous studies supporting the noxious effect that O_3 exposure can have on cutaneous tissues; however, a drawback in the research has been a lack of data derived from humans. Recently, a retrospective study from Xu et al. (2011), collecting data from almost 70,000 patients, was able to correlate the rising incidence of emergency department visits for urticaria, eczema, and contact dermatitis to an increased ambient level of ozone (O_3) (Xu et al., 2011). The evidence cited in current literature suggests the need to further investigate the harmful effect of O_3 on human skin and to evaluate possible measures to counteract its effect. For this reason, the objective of this study was to investigate whether O_3 exposure, at a level that has been observed in polluted cities (0.8 ppm), could affect skin tissue responses and whether vitamin C compound mixtures can prevent O_3-induced skin damage.

An 8-day study was conducted on 15 subjects after obtaining written informed consent. Institutional review board approval was obtained (Allendale Institutional Review Board; 7015-090-104/106-002; August 10, 2015). The subjects' forearms were randomized and divided into four zones (see Supplementary Figure S1 online): (i) MIX1 (15% L-ascorbic acid, 1% α-tocopherol, 0.5% ferulic acid [CE Ferulic, SkinCeuticals, Inc., New York, NY]), (ii) MIX2 (10% L-ascorbic acid, 2% phloretin, 0.5% ferulic acid [Phloretin CF, SkinCeuticals, Inc.]), (iii) untreated/O_3 exposed, and (iv) untreated/unexposed on the lateral forearm. The subjects' forearms were exposed to 0.8 ppm O_3 (see Supplementary Figure S2 online) for 3 hours/day for 5 consecutive days, and one punch biopsy (3-mm) sample from the four different areas was collected. Subjects were monitored for adverse events throughout the course of the study.

It is generally shown that although O_3 is not a radical species per se, its toxic effects are mediated through free radical reactions, leading to lipid peroxidation (Pryor, 1994). Known byproducts of lipid peroxidation are the α-β unsaturated aldehyde 4-hydroxynonenal (4HNE) and the 8-iso-prostaglandin-F(2α), isoprostane (Poli et al., 2008). As shown in Figure 1, after O_3 exposure, there was a significant increase of both 4HNE protein adducts (2.4-fold) and 8-iso-prostaglandin-F(2α) levels (2.1-fold) (green signal) in human skin compared with the control tissues, whereas treatment with MIX1 and MIX2 significantly prevented this effect (see Supplementary Figures S3 and S4 online). These data support previous work, in which a clear increase of 4HNE levels was observed in skin of O_3-exposed SKH-1 mice as a consequence of O_3 reaction with the outmost skin surface lipids (Valacchi et al., 2002).

Oxidative stress stimuli can cause activation of redox-sensitive transcription factors such as NF-κB. Its activation involves the dissociation of the cytosolic NF-κB/IκB complex, allowing NF-κB to translocate into the nucleus and bind to DNA for the transcription for growth factors and proinflammatory cytokines (Siomek, 2012). As shown in Figure 1, when the skin tissues were exposed to O_3, there was an evident increase in p65 signal (green fluorescence) of circa 2.4-fold. The tissues treated with both MIX1 and MIX2 clearly showed a decrease in NF-κB expression (see Supplementary Figure S5 online). The ability of O_3 to induce NF-κB activation in keratinocytes was previously demonstrated in in vitro and animal models (Valacchi et al., 2004, 2016), and we suggest that its activation is a trigger for a series of biological events leading to inflammation (Ali et al., 2016). As an example, in psoriatic epidermis, inflammatory cytokines induce a constitutive activation of NF-κB, which promotes keratinocyte hyperproliferation (Yan et al., 2015).

If activation of NF-κB is related to increased oxidative stress, being able to quench the oxidative damage induced by O_3 could prevent its activation. Indeed, O_3 exposure is able to deplete antioxidant levels in the skin (Thiele et al., 1997; Valacchi et al., 2000), and their topical application can prevent O_3-induced skin damage and NF-κB activation. These data were further confirmed by the up-regulation of cyclooxygenase-2 (COX-2), a well-known inflammatory marker regulated by NF-κB (Korbecki et al., 2013). As shown in Figure 1, O_3 induced an increase (2.7-fold) of COX-2 levels compared with unexposed skin. Treatment with MIX1 and MIX2 significantly prevented (circa 70%) the increased expression of COX-2 induced by O_3 (see Supplementary Figure S6 online). An increased COX-2 level subsequent to NF-κB activation was observed in previous studies on skin from O_3-exposed SKH-1 mice (Valacchi et al., 2004). Preventing the induction of COX-2 through the use of vitamin C compound mixtures

Abbreviations: 4HNE, 4-hydroxynonenal; COX-2, cyclooxygenase-2; MMP, matrix metalloproteinase

Accepted manuscript published online 17 February 2017

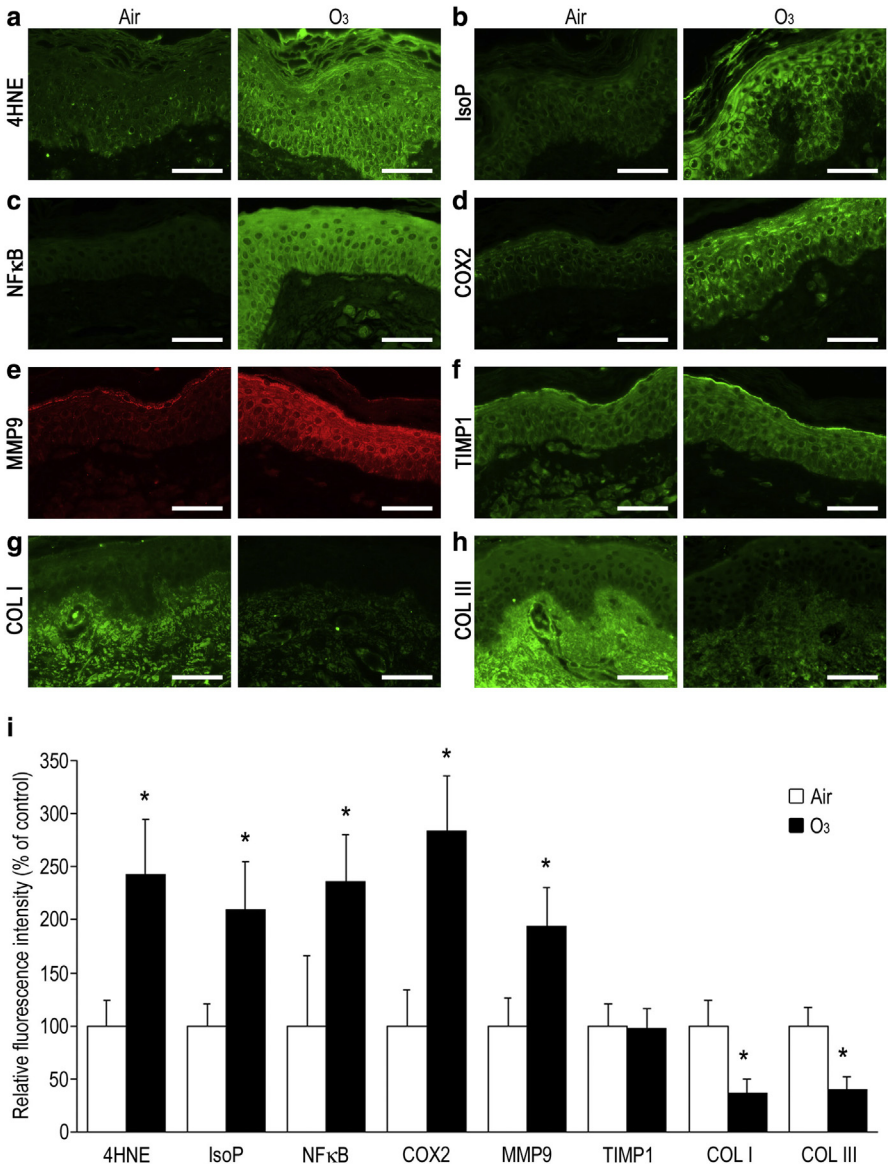

Figure 1. Exposure to unhealthy levels of ground-level ozone (0.8 ppm) damages human cutaneous tissue. Representative immunofluorescence images of human skin tissues (n = 15) stained with antibodies for (**a**) 4-hydroxynonenal (4HNE), (**b**) 8-iso-prostaglandin-F(2α) (IsoP), (**c**) NF-κB p65 subunit (NFκB), (**d**) cyclooxygenase-2 (COX2), (**e**) active form of metalloproteinase-9 (MMP9), (**f**) tissue inhibitor of metalloproteinase-1 (TIMP-1), (**g**) type I collagen (COL I), and (**h**) type I collagen (COL III). Original magnification ×630. (**i**) Immunofluorescent signal (green or red fluorescence) was semiquantified by using ImageJ software (National Institutes of Health, Bethesda, MD). Results are presented as mean ± standard deviation. *$P < 0.05$ versus air.

(MIX1 and MIX2) can have significant benefits for skin health.

Oxidative stress and inflammation, the two pathways activated by O_3, can compromise the integrity of skin by promoting connective tissue degradation via matrix metalloproteinase (MMP) expression, and O_3 has been shown to modulate the activities of enzymes involved in connective tissue turnover, such as MMP-9 (Fortino et al., 2007). In our study, MMP-9 (active form) levels were significantly increased after O_3 exposure (Figure 1) by nearly 2-fold (red signal). The treatment with MIX1 and MIX2 significantly prevented this effect. Although MMP-9 activity was altered by O_3, TIMP-1, the endogenous inhibitor of metalloproteinases, was not affected (see Supplementary Figures S7 and S8 online).

Given the important function of the collagen fibers in the maintenance of skin elasticity and resilience, collagen I and III were assessed. As shown in Figure 1, after O_3 exposure there was a significant decrease in both types of skin collagens (green signal) compared with control (−64% and −60%, respectively). Pretreatment with MIX 1 and MIX2 significantly prevented collagen marker loss. We suspect that the lower detection of collagen is mainly due to the oxidation of the proteins rather than to its degradation (see Supplementary Figures S9 and S10 online). The observed effect of O_3 on collagen and MMP levels provides evidence that O_3 exposure could also affect wound healing processes, as previously shown in animal models (Lim et al., 2006).

The oxidative effect of O_3 on human skin (depletion of vitamin E and increase in oxidized lipids), was previously described by He et al. (2006) via tape-stripping. To our knowledge, this study with biopsy analysis is the first conducted in humans that is able to show that O_3 exposure can broadly affect cutaneous tissue. Topical application of vitamin C compound mixtures appears capable of preventing the observed effects.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2017.01.034.

REFERENCES

Ali F, Khan BA, Sultana S. Wedelolactone mitigates UVB induced oxidative stress, inflammation and early tumor promotion events in murine skin: plausible role of NFkB pathway. Eur J Pharmacol 2016;786:253–64.

Fortino V, Maioli E, Torricelli C, Davis P, Valacchi G. Cutaneous MMPs are differently modulated by environmental stressors in old and young mice. Toxicol Lett 2007;173:73–9.

He QC, Tavakkol A, Wietecha K, Begum-Gafur R, Ansari SA, Polefka T. Effects of environmentally realistic levels of ozone on stratum corneum function. Int J Cosmet Sci 2006;28:349–57.

Korbecki J, Baranowska-Bosiacka I, Gutowska I, Chlubek D. The effect of reactive oxygen species on the synthesis of prostanoids from arachidonic acid. J Physiol Pharmacol 2013;64:409–21.

Lim Y, Phung AD, Corbacho AM, Aung HH, Maioli E, Reznick AZ, et al. Modulation of cutaneous wound healing by ozone: differences between young and aged mice. Toxicol Lett 2006;160(2):127–34.

Poli G, Schaur RJ, Siems WG, Leonarduzzi G. 4-hydroxynonenal: a membrane lipid oxidation product of medicinal interest. Med Res Rev 2008;28:569–631.

Pryor WA. Mechanisms of radical formation from reactions of ozone with target molecules in the lung. Free Radic Biol Med 1994;17:451–65.

Siomek A. NF-κB signaling pathway and free radical impact. Acta Biochim Pol 2012;59:323–31.

Thiele JJ, Traber MG, Tsang K, Cross CE, Packer L. In vivo exposure to ozone depletes vitamins C and E and induces lipid peroxidation in epidermal layers of murine skin. Free Radic Biol Med 1997;23:385–91.

Valacchi G, Muresan XM, Sticozzi C, Belmonte G, Pecorelli A, Cervellati F, et al. Ozone-induced damage in 3D-Skin Model is prevented by topical vitamin C and vitamin E compound mixtures application. J Dermatol Sci 2016;82:209–12.

Valacchi G, Pagnin E, Corbacho AM, Olano E, Davis PA, Packer L, et al. In vivo ozone exposure induces antioxidant/stress-related responses in murine lung and skin. Free Radic Biol Med 2004;36:673–81.

Valacchi G, van der Vliet A, Schock BC, Okamoto T, Obermuller-Jevic U, Cross CE, et al. Ozone exposure activates oxidative stress responses in murine skin. Toxicology 2002;179:163–70.

Valacchi G, Weber SU, Luu C, Cross CE, Packer L. Ozone potentiates vitamin E depletion by ultraviolet radiation in the murine stratum corneum. FEBS Lett 2000;466:165–8.

World Health Organization. WHO releases country estimates on air pollution exposure and health impact. http://www.who.int/mediacentre/news/releases/2016/air-pollution-estimates/en/; 2016 (accessed 1 November 2016).

Xu F, Yan S, Wu M, Li F, Xu X, Song W, et al. Ambient ozone pollution as a risk factor for skin disorders. Br J Dermatol 2011;165:224–5.

Yan S, Xu Z, Lou F, Zhang L, Ke F, Bai J, et al. NF-κB-induced microRNA-31 promotes epidermal hyperplasia by repressing protein phosphatase 6 in psoriasis. Nat Commun 2015;6:7652–66.

Increased Risk of Cutaneous and Systemic Infections in Atopic Dermatitis—A Cohort Study

Journal of Investigative Dermatology (2017) **137,** 1375–1377; doi:10.1016/j.jid.2017.01.030

TO THE EDITOR

Atopic dermatitis (AD, also known as atopic eczema or eczema), is characterized by skin barrier and immunologic dysfunction. Viral and bacterial superinfection of cutaneous lesions, including eczema herpeticum and *Staphylococcus aureus* in patients with severe disease is well documented (Ong and Leung, 2016; Weidinger and Novak, 2016). Whether the general population of patients with AD has an increased risk of these and other types of infections because of an impaired skin barrier and/or immunologic dysfunction is unclear.

A recent meta-analysis of genome-wide association studies identified mutations in genes thought to play roles in the regulation of innate and adaptive immunity, in addition to established barrier function susceptibility loci such as filaggrin (Paternoster et al., 2015). Investigations of skin physiology suggest that differences in barrier function are identifiable very early in infancy and are highly predictive of the development of AD (Kelleher et al., 2015). We therefore hypothesized that individuals who develop AD are at increased risk of infections because of underlying genetically influenced immune and barrier dysfunction. The objective of our study was to determine if there was an association between AD and multiple common cutaneous and noncutaneous infections.

We performed a cohort study using The Health Improvement Network, a medical records database that is representative of the UK general population (Seminara et al., 2010). Ethics approval for this study was obtained from The Health Improvement Network Scientific Review Committee and the University of Pennsylvania Institutional Review Board. We included 3,112,617 individuals registered before age 18 years who were followed for a mean of 13.7 years (95% confidence interval = 13.6, 13.7). We identified subjects with AD based on the presence of at least one of any of the following diagnostic codes on two different visits, as is common practice in studies of chronic conditions using electronic health data (Herrett et al., 2010): atopic dermatitis and related conditions (M11.00), atopic dermatitis/AD (M111.00), and atopic dermatitis not otherwise specified (M11z.00). The prevalence of AD was 14.4% (95% confidence interval = 14.4–14.4).

We examined the prevalence of multiple common cutaneous and noncutaneous infections (warts, dermatophyte infection, impetigo, molluscum contagiosum, otitis media, pneumonia, and streptococcal throat infection; codes available in Supplementary Table S1 online). We found that all of the infectious illnesses we had determined to test a priori were more prevalent in those with AD. Using multilevel mixed-effects logistic regression, we examined the odds of each infectious outcome at any time point and found that the strength of association for cutaneous infections varied from a 55% increased odds of impetigo to a 3-fold increased odds of

Abbreviation: AD, atopic dermatitis

Accepted manuscript published online 12 February 2017; corrected proof published online 8 April 2017