Involvement of 4-hydroxy-2-nonenal in pollution-induced skin damage

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Abstract
The effects of environmental insults on human health are a major global concern. Some of the most noxious pollutants that humans are exposed to include ozone (O₃), particulate matter (PM), and cigarette smoke (CS). Since the skin is the first line of defense against environmental insults, it is considered one of the main target organs for the harmful insults of air pollution. Thus, there is solid evidence that skin pathologies such as premature aging, atopic dermatitis (AD), and psoriasis are associated with pollutant exposure; all of these skin conditions are also associated with an altered redox status. Therefore, although the mechanisms of action and concentrations of O₃, PM, and CS that we are exposed to differ, exposure to all of these pollutants is associated with the development of similar skin conditions due to the fact that all of these pollutants alter redox homeostasis, increasing reactive oxygen species production and oxidative stress. A main product of oxidative stress, induced by exposure to the aforementioned pollutants, is 4-hydroxy-2-nonenal (HNE), which derives from the oxidation of ω-6 polyunsaturated fatty acids. HNE is a highly reactive compound that can form adducts with cellular proteins and even DNA; it is also an efficient cell signaling molecule able to regulate mitogen-activated protein kinase pathways and the activity of redox-sensitive transcription factors such as Nrf2, AP1, and NFκB. Therefore, increased levels of HNE in the skin, in response to pollutants, likely accelerates skin aging and exacerbates existing skin inflammatory conditions; thus, targeting HNE formation could be an innovative cosmeceutical approach for topical applications.

KEYWORDS
ozone, particulate matter, cigarette smoke, peroxidation

1 | INCREASING AIR POLLUTION AND IMPLICATIONS FOR HUMAN SKIN HEALTH

Ubiquitous exposure to environmental insults is a major global concern for public health. Over the past 30 years, the damaging effects of pollutants on cutaneous tissue have become an area of growing research interest, also in view of increased environmental pollution. Indeed, a recent search in PubMed using the keywords “skin and pollution” reveals...
more than 2000 papers related to this topic (https://www.ncbi.nlm.nih.gov/pubmed/?term=skin+pollution). Until the year 2000, only a few articles were published on this issue; however, in the last 20 years, interest in this field has grown exponentially, reaching approximately 100 manuscripts per year. This increased interest is related not only to the proven and well-established worsening of air quality but also to the evidence of a direct correlation between skin pathologies and air pollution exposure.\(^1\)\(^-\)\(^4\) Indeed, the skin is the first line of defense because it is the interface between the environment and our body. Therefore, cutaneous tissue is considered, along with eyes, lung, brain, and digestive tract, as one of the main target organs for the harmful insults of air pollution.\(^5\)

1.1 Cutaneous tissues as a gateway for the outdoor pollutants

Growing evidence demonstrates that the skin also constitutes a route of entry for ambient pollutants in the human body, promoting considerable systemic consequences in almost all internal organs.\(^6\)\(^-\)\(^8\) In a very elegant series of experiments, Weschler et al. (2015) were able to demonstrate the ability of volatile organic compounds (VOCs), specifically diethyl phthalate (DEP) and di(n-butyl)phthalate (DNBP), to be absorbed by the skin. Surprisingly, the amounts of phthalate metabolites found in urine were similar when the subjects were exposed to pollutants either by breathing or by dermal exposure, suggesting that the ability of our body to absorb pollutants from the outdoor environment by the cutaneous tissues is similar, if not more efficient than the respiratory tract. Even more striking is the finding that dermal absorption of VOCs increases tremendously with age; indeed, subjects over 60 years old had five times higher levels of the phthalates in urine compared to 30-year-old subjects.\(^8\)

Interestingly, a role of clothing in influencing the cutaneous uptake of harmful compounds from polluted air has been investigated by several groups\(^8\)\(^-\)\(^11\); surprisingly, clean clothes are only able to protect the skin by about 20–30% from pollution absorption, eventually being a source of pollutant accumulation, and increasing cutaneous uptake if not changed daily.\(^9\)\(^,\)\(^10\)

2 POLLUTION AND SKIN PATHOLOGIES

The use of the word “pollution” can be misleading given that there are several different pollutants that can affect our health. In addition, not all of them have the same concentration in the air and the same mechanism of action. Based on their chemical and physical properties as well as their sources, the United States Environmental Protection Agency has identified the most common air pollutants, also known as “criteria air pollutants”, as ozone (O\(_3\)), particulate matter (PM), carbon monoxide (CO), lead, sulfur dioxide (SO\(_2\)), and nitrogen dioxide (NO\(_2\)) (https://www.epa.gov/criteria-air-pollutants). Clear evidence of the correlation between each single pollutant and skin disorders has not yet been established; however, the noxious effects of O\(_3\), cigarette smoke (CS), and PM have been well demonstrated, as described in the following section.

Overall, there is solid evidence that pathologies such as atopic dermatitis (AD), psoriasis, acne, and, in some cases, also skin cancer can be associated with pollutant exposure. In particular, exposure to O\(_3\), PM, NO\(_2\), and CS has been demonstrated to be associated with cutaneous pathologies. The seminal epidemiological work by Xu et al. (2011) demonstrated the association of these pollutants with cutaneous pathologies by analyzing the association between emergency-room (ER) visits for skin conditions and levels of air pollutants including O\(_3\), PM\(_{10}\), SO\(_2\), and NO\(_2\). During 2 years of sampling, over 68,000 visits to the ER for skin disorders were recorded, and a clear correlation between O\(_3\) concentration and cutaneous issues was demonstrated. In particular, the authors underlined how several skin conditions such as urticaria, eczema, contact dermatitis, rash/other nonspecific eruption, and infected skin diseases were exacerbated when the subjects were exposed to increased levels of this pollutant.\(^12\) These data suggest a role of O\(_3\) in inducing inflammatory skin pathologies. Another more recent publication has further examined the association of short-term changes in air quality with emergency department (ED) visits for urticaria in Canada. A total of 2905 ED visits were analyzed, and a positive and significant correlation was observed between air quality levels and ED visits for urticaria, confirming that air pollution can affect skin physiology.\(^13\)

AD is a chronic and recurrent cutaneous inflammatory disease that begins in the early stage of life. The pathogenesis of AD is usually linked to skin barrier alteration and immune dysregulation.\(^14\)\(^,\)\(^15\) Indeed, changes in the stratum corneum (SC) composition, which is the outermost layer of the skin, can facilitate the penetration of allergens that can then be associated with the development of AD.\(^15\) As opposed to the outside-in model of AD pathogenesis,\(^16\) the inside-out theory suggests that Th2 cytokines are able to modulate the expression of proteins present in the SC, thereby disrupting the skin barrier.\(^17\)

Although there is still existing controversy between these two theories, one fact is clear; the perturbation of the skin barrier plays a key role in AD, and this perturbation can be induced by environmental pollutant exposure. For instance, there is now evidence that air pollution influences the prevalence of AD. In a fairly recent study, it was shown that, in a population of almost 5000 children from France, there was a direct correlation between the development of eczema and pollution levels (PM\(_{10}\) and NO\(_x\)).\(^18\) In addition, a study conducted in the Munich metropolitan area revealed a strong
positive association between the distance to the nearest main road and eczema; in particular, it was found that NO\textsubscript{2} was positively associated with eczema in children exposed to traffic-related air pollution.\textsuperscript{19} In a more recent work, PM\textsubscript{10} and NO\textsubscript{2} exposure during the first trimester of pregnancy was associated with the development of infantile AD.\textsuperscript{20} It has also been demonstrated that maternal smoking during pregnancy and/or in the first year after birth is a major risk factor for the development of AD among children aged between 6 and 13 years.\textsuperscript{21} Similarly, fetal tobacco smoke exposure during the third trimester of pregnancy was positively associated with a higher cumulative incidence of atopic eczema/dermatitis syndrome in exposed infants in a Japanese study; the authors suggest that maternal smoking might induce epigenetic changes in the fetal allergen-specific immune responses, promoting development of AD.\textsuperscript{22}

Thus, the evidence that exposure to environmental tobacco smoke during early childhood can predispose children to later development of AD has been documented, but it is still not clear whether current smokers develop AD. This point was well clarified in the paper by Lee et al., in which, among 83 patients diagnosed with adult-onset AD, more than 50% were current smokers, and about one-third have smoked in the past.\textsuperscript{23} This study strongly supports the idea of the association between current smoking and the development of adult-onset AD, as well as a correlation between exposure to CS and AD in nonsmokers.

Besides eczema and AD, psoriasis is another inflammatory-related skin disease that appears to be associated with air pollution. Indeed, it has been proposed that exposure to pollutants such as PM,\textsuperscript{24} CS,\textsuperscript{25} or O\textsubscript{3}\textsuperscript{26} can activate the aryl hydrocarbon receptor (AhR), and this can further activate Th17 cells\textsuperscript{27}; the main cells involved in psoriasis and present in psoriatic lesions. Although there is controversy in the link between CS and psoriasis, a recent study with over 17 million patients over the age of 20 years that were followed for 8 years was able to clearly show the positive correlation between risk of psoriasis and smoking period.\textsuperscript{28} In addition, this correlation was stronger for subjects that smoked more than two packs per day and much lower for 0.5 pack smokers.\textsuperscript{28}

Interestingly, CS has been also connected with the development of acne,\textsuperscript{4} another multifactorial skin disease. Acne is usually characterized by increased sebum production, abnormal keratinization of the pilosebaceous duct, and inflammation driven by the presence of Propionibacterium acnes. It has been shown that these processes are induced by an altered redox status, which pollutant exposure can generate.\textsuperscript{29} Indeed, the ability of PM, CS, and even O\textsubscript{3} to increase reactive oxygen species (ROS) production and activate a cascade of events, leading to increased OxInflammation, has been well proven. In a recent work, our laboratory was able to show that CS-induced OxInflammation hampers the ability of sebocytes to uptake cholesterol via oxidation of an important skin receptor, the scavenger receptor class B type I (SRB1).\textsuperscript{30} This pathway has also been observed in other skin models, such as keratinocytes and 3D skin equivalents, and is related not only to CS but also to PM and O\textsubscript{3} exposure\textsuperscript{31–33} (GV unpublished data).

In addition to the aforementioned skin conditions, environmental changes, due to rapid industrialization and urbanization of the last few decades, are suspected to be the main drivers of the increased incidence of skin pigmentation in geographic regions with very heavy pollution, such as India and South East Asia. Indeed, pollutants, such as PM and polycyclic aromatic hydrocarbons (PAH), due to their ability to enter the skin via nanoparticles, also appear to be important risk factors for facial hyperpigmentation disorders, specifically melasma.\textsuperscript{34} Exposure to PM has also been implicated in the development, persistence, and exacerbation of other cutaneous conditions, such as AD, acne, psoriasis, skin aging, androgenetic alopecia, and skin cancer.\textsuperscript{35}

In conclusion, ambient air pollutants, such as PM, CS, or O\textsubscript{3}, seem to be involved in the pathogenesis of inflammatory skin diseases (e.g., AD, acne, and psoriasis) via a common denominator, that is, through enhancing oxidative stress and proinflammatory mediators (OxInflammation phenomena)\textsuperscript{36} (Figure 1).

### 3 | MECHANISMS INVOLVED IN POLLUTION AFFECT CUTANEOUS TISSUES

#### 3.1 | Atmospheric skin damage

Although all pollutants are able to induce OxInflammation as the final outcome of their harmful effects, it is interesting to note that each of them can affect human skin physiology through different mechanisms of action. Based on their chemical and physical characteristics, only some air contaminants are able to penetrate through the layers of the skin, reaching the dermis. Therefore, the potential damaging effects and the way by which each pollutant impacts skin structure and function differs substantially.

O\textsubscript{3} is a small molecule and strong oxidizing agent that directly acts on the surface of cutaneous tissue, disseminating its detrimental effects into the deeper epidermal layers through the generation of a cascade of ozonation products. Although it is not a radical species per se, O\textsubscript{3} is able to oxidize components of the cell membrane, mainly lipids, generating classical radical species such as hydroxyl radicals that, in turn, drive the production of cytotoxic, nonradical species including aldehydes. Due to its high reactivity and chemical and physical properties of low aqueous solubility within the skin, O\textsubscript{3} is not able to reach and directly damage live epidermal and dermal
cells. In fact, it is well proven that this pollutant is entirely consumed through reaction with skin surface lipids and the intercellular lipids of the SC.37

As a mechanically protective and flexible structure mainly constituted of anucleated “dead” corneocytes, the outermost stratum of our skin is enriched by sebaceous and intercellular lipids including squalene, triglycerides, ceramides, free fatty acids, wax monoesters, and cholesterol. Since all of these lipids are easily prone to oxidation, as a natural consequence, their reaction with O3 can generate several secondary messengers able to trigger signaling cascades across the different layers of skin, leading to prooxidative and proinflammatory processes.38–40

For instance, in a recent study, it has been shown that O3 exposure increased levels of HNE in human skin, and this correlated with increased proinflammatory markers such as COX2 and NFκB.41 Interestingly, the levels of HNE followed a clear gradient pattern, with high levels in the upper epidermis and lower levels in the dermis, suggesting that the effect of O3 on the skin is indeed mediated by oxidation products generated mainly in the upper layers of the epidermis.41 Therefore, it is possible to claim that the effect of O3 on cutaneous tissues is a consequence of its reaction with the lipids present in the SC.

This concept was first advanced by Pryor et al.42 in relation to the respiratory tract, suggesting that exposure of noncellular constituents of surface epithelial cells to O3 is capable of generating potentially toxic peroxidation products. Extrapolation of this concept to cutaneous tissues suggests that O3 reacts directly with the SC lipids that contribute to the cutaneous tissue protective barrier,39 generating products that are able to penetrate the SC and target keratinocytes. It is concluded that O3 not only affects cutaneous “antioxidant” levels and oxidation markers in the SC but also induces cellular responses in the deeper layers of the skin.

Low-molecular-weight antioxidants are present in high concentrations, especially in the epidermis. Oxidative stress can overwhelm skin defenses and increase the formation of oxidized cell components. Topical exposure to tropospheric O3 induces oxidative imbalances in the skin. Oxidative damage to the SC may result in barrier perturbation and in the production of lipid oxidation products that can act as “second messengers” in the deeper layers of the skin, which, in turn, elicits repair responses and/or the induction of defense proteins such as NRF2 and/or heat shock proteins (HSPs). Oxidative injury to the outermost layers of the skin can initiate localized inflammatory responses, resulting in the recruitment of phagocytes and their tightly regulated, cell-specific NAD(P)H-oxidase systems for generating oxidants, further amplifying the oxidative stress damage.41

As of today, the potential overall mechanism by which O3 is able to affect skin has been described. It is generally understood that the toxic effects of O3, although it is not a radical species per se, are mediated through free radical reaction either directly by the oxidation of biomolecules to give classical radical species (hydroxyl radical) or by driving the radical-dependent production of cytotoxic, nonradical species (aldehydes). Furthermore, the formation of oxidation products, characteristic of damage from free radicals, has been shown to be prevented by the addition of the vitamins E and C. O3 is not able to penetrate the SC, so it first interacts with the lipids present in the outermost layer of the skin, leading to the generation of a number of bioactive species. Our lab together with other recent works has provided some evidence that these bioactive compounds are likely to penetrate the underlying cutaneous tissues, as demonstrated by the presence of several proinflammatory markers in the deeper layer of the skin.40 It can be suggested that reaction with the well-organized interstitial lipids and protein constituents of the outermost SC barrier, and diffusion of bioactive products from this tissue into the viable layers of the epidermis, may represent a contribution to the development/exacerbation of skin disorders associated with O3 exposure. Indeed, once these “mediators” are able to reach live cells (keratinocytes, fibroblasts, etc.), they can induce a cellular defensive and

**FIGURE 1** Consequences of 4-hydroxy-2-nonenal (HNE) production in response to pollutants. Exposure of the skin to ozone, cigarette smoke, and particulate matter induces lipid peroxidation and production of HNE. This product of lipid peroxidation can form covalent bonds with the histidine, cysteine, and lysine residues of proteins, such as cytochrome c, through Michael addition, generating ROS and oxidative stress, which can promote/exacerbate cutaneous conditions such as psoriasis, premature aging, and AD. ROS, reactive oxygen species.
inflammatory response that leads to an inflammatory/oxidative vicious cycle, OxInflammation. This, unless quenched by endogenous or exogenous mechanisms, will damage the skin and compromise its barrier functions, contributing to extrinsic skin aging.

3.2 | Mechanisms involved in CS effects on skin

CS is a highly complex aerosol composed of more than 4700 chemicals and consists of a gas phase and a particulate phase. Mainstream smoke (the combination of inhaled and exhaled smoke after taking a puff of a lit cigarette) includes particulates suspended in a gaseous phase. It is widely recognized that CS contains high levels of prooxidants, with more than $10^{14}$ low molecular weight carbon- and oxygen-centered radicals per puff present in gas-phase smoke. Sidestream smoke goes into the air directly from a burning cigarette and is the main component of second-hand smoke. The chemical constituents of sidestream smoke are different from those of directly inhaled (mainstream) CS; it has been shown that inhaled sidestream CS is approximately four times more toxic per gram of total particulate matter than mainstream CS. Furthermore, sidestream condensate, compared to mainstream, is about three times more toxic per gram and two to six times more tumorigenic per gram. The gas/vapor phase of sidestream smoke is responsible for most of the sensory irritation and respiratory tract epithelium damage.

As mentioned above, the toxic effect of CS on the skin has been well demonstrated. Exposure to CS can result in impaired wound healing, development of squamous cell carcinoma, oral cancer, acne, psoriasis, eczema, hair loss, and premature skin aging. Epidemiological studies strongly correlated CS to premature skin aging. Moreover, the obvious esthetic damage of the skin by CS was well documented by Dr. Model more than 30 years ago, who defined the so-called “smoker’s face,” characterized by gray skin (smoker’s melanosis) and deep wrinkles (smoker’s wrinkle). Indeed, wrinkle formation is a typical feature associated with tobacco smoking. CS is able to affect skin aging by activating MMPs in the connective tissues. For instance, MMP-1 induces the degradation of both collagen and elastic fibers. In addition, production of the procollagen types I and III is affected by CS, while MMP-1 and MMP-3 are strongly induced. The mechanisms involved in CS-induced skin aging remain unresolved, although it is believed that activation of the AhR signaling pathway contributes to this effect.

CS contains water-insoluble PAHs, which have been linked to activation of the AhR signaling pathway. AhR is involved in the regulation of development, hypoxia signaling, and circadian rhythms, and belongs to a family of proteins that reside in the cytoplasm in an inactive complex with accessory proteins. Once activated, AhR dissociates from some of the proteins in the inactive complex and translocates to the nucleus, where it dimerizes with Arnt. The AhR/Arnt heterodimer activates the transcription of xenobiotic-metabolizing genes, some of which encode proteins involved in growth control, cytokines, nuclear transcription, and regulators of extracellular matrix proteolysis. Therefore, the AhR pathway may be involved in the effects of tobacco smoke on skin. In support of this idea, CS increased MMP-1 mRNA induction in primary keratinocytes and fibroblasts, and AhR knockdown abolished this effect, suggesting the involvement of AhR activation in extrinsic skin aging induced by CS.

In addition to premature aging, CS has also been linked to psoriasis. As previously mentioned, a recent study of over 17 million subjects demonstrated a positive correlation between smoking and psoriasis, which correlated with how many packs per day the subjects smoked. The molecular basis of this effect is likely due to increased oxidative stress in the skin induced by CS. In fact, our lab has demonstrated that CS exposure in keratinocytes increases NAPDH oxidase activity as assessed via p47 and p67 membrane translocation, resulting in increased H$_2$O$_2$ levels and mitochondrial superoxide production. We also observed that CS exposure in keratinocytes increases the levels of HNE and acrolein adducts. We believe that increased NAPDH oxidase activity is due to increased production of HNE adducts in response to CS exposure, since Yun et al. (2005) demonstrated that HNE production is able to directly activate NOx. Moreover, the increased levels of superoxide anion or H$_2$O$_2$ produced by NOx can regulate the AhR signaling pathway, connecting AhR activation to oxidative stress responses. It is also possible that the AhR transcription factor itself can be modified by HNE. Since increased oxidative stress in the skin has been associated with premature aging, the ability of CS to induce oxidative damage likely contributes to premature aging.

3.3 | Mechanisms involved beyond PM-induced skin damage

PM is a complex, heterogeneous mixture of particles, which vary in size, number, surface areas, concentrations, and chemical composition. PM particles can be emitted directly from sources like fossil-fuel combustion as well as generated from gases through reactions involving other pollutants. PM particles can be either liquid, solid, or liquid surrounding a solid core and can be composed of organic chemicals, metals, and soil or dust particles, as well as nitrates and sulfates, which can be further categorized into different particles based on their sizes, such as PM$_{10}$, PM$_{2.5}$, and ultrafine particles (UFPs). Coarse particles have a diameter of 2.5 to 10 μm (PM$_{10}$) and can be generated by farming, mining, and construction. Fine particles have a diameter of 2.5 μm or less (PM$_{2.5}$) and can be
generated by power plants, fuel refineries, fuel combustion, cars, and wildfires. There are also UFPs with diameters less than 0.1 μm or 100 nm that can be generated by diesel and gasoline fuel combustion from cars, aircrafts, and ships. These particles can differ not only in their size but also in their effects on human health. A study conducted in 2000 demonstrated that PM$_{2.5}$ particles that are generated by combustion sources were associated with increased daily mortality. Inhalation of PM$_{2.5}$ results in increased plaque deposits in arteries, promoting development of atherosclerosis as well as increased risk of heart attacks.

Most studies on the effects of PM particles on human health have focused on the negative effects of inhaling these particles such as asthma, lung cancer, cardiovascular disease, premature death, and premature delivery and birth defects in babies. However, epidemiological studies indicate that PM can promote premature skin aging and exacerbate preexisting skin diseases. Exposure to PM is associated with progression of AD in children, and an improvement in air quality resulted in decreased prevalence and severity of AD.

The mechanisms involved in PM-associated skin disorders result from increased oxidative stress due to PM exposure. PMs can move through the skin through hair follicles or transdermally, generating oxidative stress. PAHs are components of UFPs that can be absorbed through the skin and eventually damage the mitochondria, resulting in intracellular ROS production. These damaged mitochondria produce superoxide anions, which can be converted into H$_2$O$_2$ that can then undergo the Fenton reaction to produce hydroxyl radicals, resulting in increased ROS and activation of redox-sensitive transcription factors, such as AP1 and NFκB. In addition, interactions between PM particles and surfaces can result in extracellular ROS production, again resulting in the activation of redox-sensitive transcription factors AP1 and NFκB. The consequences of oxidative stress result in antioxidant depletion, lipid peroxidation, and DNA damage. In support of this idea, our lab has demonstrated that exposure to PM particles induces nuclear translocation of NFκB, increases levels of HNE, and promotes DNA damage in human biopsies. The consequences of increased oxidative stress in response to PM exposure result in the exacerbation of preexisting skin diseases and premature skin aging.

### 3.4 HNE: the trigger for pollution-induced skin OxInflammation

HNE derives from the oxidation of ω-6 polyunsaturated fatty acids (PUFAs), essentially arachidonic and linoleic acid, that is, the two most represented fatty acids in biomembranes; for a more in-depth view of HNE production, see. In the context of pollutants and skin, HNE has been shown to be produced in the cutaneous tissues after exposure to O$_3$, PM, and CS. As previously mentioned, O$_3$ immediately interacts with the PUFAs present in the upper layers of the epithelium, oxidizing these lipids and forming unstable peroxides that can then lead to the formation of HNE. The mechanism by which PM is believed to induce the production of HNE is less direct; the transition metal constituents of PM (Fe, Zn, Ni, etc.) are believed to undergo Fenton or Fenton-like reactions, generating ROS like OH$^-$. In addition, as previously mentioned, PAHs, which are components of PM that are highly lipophilic, can localize to the mitochondria and promote the generation of mitochondrial-produced ROS. These increased levels of ROS can promote the oxidation of ω-6 PUFAs, generating HNE. CS is also believed to promote the generation of HNE through increased ROS as our lab has shown that CS exposure in keratinocytes increases NAPDH oxidase activity resulting in increased H$_2$O$_2$ levels.

HNE is an unusual compound containing three functional groups that in many cases act in concert, explaining its high reactivity (Figure 2). There is, first of all, a conjugated system consisting of a C=C double bond and a C=O carbonyl group in HNE. The hydroxyl group at carbon 4 contributes to reactivity both by polarizing the C=O bond and by facilitating internal cyclization reactions, such as thioacetal formation. HNE is an amphiphilic molecule; in fact, it is water soluble and also exhibits strong lipophilic properties. Consequently, HNE tends to concentrate in biomembranes, where phospholipids, like phosphatidylethanolamine, and proteins, such as transporters, ion channels, and receptors, quickly react with HNE. In addition, since it is a highly electrophilic molecule, it easily reacts with low molecular weight compounds, such as glutathione, and at higher concentrations with DNA (Figure 2). Because of its electrophilic nature, HNE can form adducts with cellular protein nucleophiles. Indeed, the reactivity of HNE explains its potential involvement in the modulation of enzyme activity, signal transduction, and gene expression. Besides being a product of oxidative stress, HNE is also an efficient cell signaling molecule able to modulate the expression of several genes; therefore, it may influence important cellular functions such as cell growth, differentiation, and apoptosis. An increasing amount of literature indicates that HNE, depending on the concentrations, can potently activate stress response mechanisms, such as mitogen-activated protein kinases (MAPKs), detoxification mechanisms, and inflammatory responses, contributing to cell survival against cytotoxic stress. Furthermore, HNE may modulate redox-sensitive transcription factors such as nuclear factor-kappa B (NFκB), activator protein-1 (AP1), and nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Moreover, its proven interaction with a variety of enzymes and kinases variously involved in cell signaling strongly support its important role in pathophysiology as a cell signaling messenger (Figure 2).
Once formed, under physiological conditions, HNE is rapidly degraded in mammalian cells by multiple enzymatic pathways. The best characterized of these enzymes include the glutathione-S-transferases (GSTs), aldehyde dehydrogenase, and alcohol dehydrogenase. GSTs catalyze the conjugation of reduced glutathione (GSH) to HNE via Michael addition at the C-3 carbon, thereby preventing further nucleophilic addition to this toxic compound. Aldehyde dehydrogenase catalyzes the oxidation of HNE to the innocuous 4-hydroxy-2-nonenoic acid, while alcohol dehydrogenase catalyzes reduction of the terminal aldehyde to its alcohol, yielding the reactive metabolite 1,4-dihydroxy-2-nonene (DHN). Another enzyme involved in the metabolism of HNE is aldose reductase, a member of the aldo–keto reductase superfamily. This enzyme has been shown to catalyze the reduction of the GSH conjugate of HNE, leading to DHN–GSH.80,81

The half-life of HNE has been studied in several cell types, in subcellular organelles, and even in whole organisms. Liver tissue generally has the highest capacity to metabolize HNE, while in other cells, the metabolism of HNE is not so fast, but still very efficient.80,81 Usually, HNE, even at very high lipid peroxidation rates, cannot accumulate in an unlimited manner. However, compared with other oxidants, such as most types of ROS, HNE is chemically better suited for its role as a signaling molecule because of its longer half-life and thus greater range of diffusion and a higher selectivity for reaction with specific targets. Therefore, despite the fact that humans have developed several enzymatic systems to rapidly detoxify HNE molecules, HNE can escape detoxifying processes and migrate from the site of origin to other intracellular sites, reacting rapidly with biological macromolecules, especially proteins to form HNE protein adducts (PAs).83

HNE PAs are physiological constituents of mammalian organisms. They are easily detectable in peripheral blood, where they primarily involve albumin, transferrin, and immunoglobulins, and also proteins related to blood coagulation, lipid transport, blood pressure regulation, and protease inhibition.80,81 Nevertheless, as proteins play an important role in the normal structure and function of cells, oxidative modifications promoted by increased HNE levels may greatly alter their structure. These protein alterations may subsequently lead to loss of normal physiological cell functions and/or may lead to abnormal function of the cell and eventually to cell death. For instance, HNE can modify mitochondrial proteins such as cytochrome c, impairing mitochondrial metabolism.84 HNE PAs also contribute to the pool of damaged enzymes, which increases during aging and in several pathological states.85

As previously mentioned, HNE can regulate a variety of normal cell processes including cell growth, differentiation,
and apoptosis. This is likely because HNE can activate a variety of signal transduction pathways including the Erk pathway, p38MAPK, c-Jun N-terminal kinases (JNK) pathway, and epidermal growth factor receptor (EGFR) pathway. In addition, it can upregulate the extrinsic and intrinsic apoptotic pathways. Moreover, it can regulate the activity of critical transcription factors involved in OS responses such as Nrf2 and peroxisome-proliferator-activated receptors (PPARs). For instance, HNE can induce Nrf2 activation by modifying its inhibitor Keap1, releasing Nrf2 for nuclear export. Moreover, it can enhance the DNA-binding activity of AP1 and both negatively and positively regulate NFκB. Because of these findings, it is believed that HNE can be causally involved in many of the pathophysiological effects associated with oxidative stress in cells and tissues, especially for the skin due to its rich concentration of omega-6 fatty acids. Most studies assessing HNE production in the skin have primarily focused on assessing the levels of HNE as a marker of oxidative stress; therefore, the role of HNE in skin biology remains largely unexplored.

5 | PATHOLOGICAL EFFECTS OF POLLUTION-INDUCED HNE IN THE SKIN

Indeed, the presence of HNE adducts in the skin after pollution exposure has been well documented and in several cases linked to skin aging, making this marker a possible common mediator of skin oxidative damage. For instance, HNE levels were increased after O3 exposure in both 2D and 3D models and in human skin biopsies after 5 days of O3 exposure. These results are in line with previous animal work, wherein hairless mice exposed to O3 exhibited a clear increase of HNE PAs in the epidermis. Interestingly, cutaneous HNE levels were also significantly higher after O3 exposure in old animals, compared to young animals, in a wound healing study, suggesting their role in delaying cutaneous wound closure in aged mice, possibly via the aberrant activation of metalloproteinases (MMPs). O3 is not the only pollutant that induces the formation of cutaneous HNE in the skin; indeed, similar effects have been observed after both CS and PM exposure. Our group was able to demonstrate the formation of HNE PAs in both keratinocytes and reconstituted human epidermis tissues (RHEs) after CS and PM exposure. The formation of HNE PAs, as mentioned before, leads to the covalent modification of proteins, which can be subsequently ubiquitinated and degraded. Therefore, a consequence of HNE adduct formation is the loss of important cellular proteins, such as SRB1, which we observed in both keratinocytes and sebocytes. In a very recent work by Verdin et al. (2019), the ability of UFPs to increase HNE levels in RHEs was also observed, suggesting a role of HNE in regulating skin differentiation and cornification.

In the skin, we believe that the physiological effects of high levels of HNE in response to pollutant-induced oxidative stress results in the development/exacerbation of premature aging, psoriasis, and AD. Several studies have demonstrated a role for HNE in skin aging and in skin color. In addition, HNE PAs have been detected in photodamaged skin elastosis. The

FIGURE 3 4-Hydroxynonenal (HNE) as a pollutant-induced signaling mediator. Exposure of the skin to ozone, cigarette smoke, and particulate matter induces lipid peroxidation and subsequent production of HNE, resulting in the Michael addition of HNE to protein products such as cytochrome c, which results in ROS production via mitochondria, and Keap1, which releases Nrf2 from sequestration. In addition, HNE activates NADPH oxidase, again resulting in the generation of ROS and oxidative stress, which can activate NfκB and AhR. HNE can also activate MAPK pathways, ultimately resulting in the activation of HSPs and transcription factors NFB and AP1. Moreover, modification of cytochrome c by this signaling mediator can induce caspase activation and PARP1 cleavage. ROS, reactive oxygen species.
effects of HNE in skin aging, associated with pollutant exposure, could be due to its ability to degrade collagen due to altering MMP levels, which are transcriptionally regulated by NFκB. A general review of the role of HNE in aging can be found here. Increased HNE levels have been also detected in skin samples from psoriatic patients as well as in the cases of AD. Since inflammation is the primary cause of both of these skin conditions, it is likely that the ability of HNE to regulate the activity of the key proinflammatory mediator NFκB contributes to these conditions.

6 | CONCLUSION

Cutaneous exposure to pollutants is associated with the development/exacerbation of premature aging, psoriasis, and AD conditions, and these effects are believed to be mediated by pollutant-induced oxidative stress. Since oxidative stress results in the production of HNE, it is no surprise that pollutant exposure results in increased levels of HNE in the epithelium. However, HNE is not just a marker of oxidative stress but also an important signaling mediator that plays its roles in a variety of cellular pathways including apoptosis, differentiation, cell growth, and migration through the formation of HNE adducts as well as modulation of signal transduction pathways. Thus, being able to prevent cutaneous HNE formation could be a possible innovative cosmeceutical approach for future topical applications targeting the aforementioned skin conditions (Figure 3).

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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