4HNE key mediator of pollution-induced skin damage

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ABSTRACT

Due to increasing environmental pollution, the effects of environmental insults on human health is a major global concern. Among the most noxious pollutants there are ozone (O_3), particulate matter (PM) and cigarette smoke (CS). Since the skin is the first line of defense for our bodies against environmental insults, it is is considered as 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 O_3, PM, and CS that we are exposed to differ, all of these are associated with the development of similar skin conditions due to the fact that all of these pollutants alter redox homeostasis, increasing ROS production and oxidative stress. A main product of oxidative stress, induced by exposure to the aforementioned pollutants, is 4-hydroxy-2-nonenal (4HNE), which derives from the oxidation of ω-6 PUFAs. 4HNE is a highly reactive compound that can form adducts with cellular proteins and even DNA; it is also an efficient cell signalling molecule able to regulate MAPK kinase pathways and the activity of redox-sensitive transcription factors such as Nrf2, AP1, and NFκB. Therefore, increased levels of 4HNE in the skin, in response to pollutants, likely accelerates skin aging and exacerbates existing skin inflammatory conditions; thus, targeting 4HNE formation could be an innovative cosmeceutical approach for topical applications.

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 has become an area of growing research interest, also in view of the increases in 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; in the last 20 years, interest in this field has grown exponentially, reaching circa 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, as the interface between the environment and our body, the skin is the first line of defense. 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].

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 VOCs, specifically diethyl phthalate (DEP) and di(n-butyl) phthalate (DnBP), to be absorbed by the skin. Surprisingly, the amounts of phthalates 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 5 times higher levels of the phthalates in urine, compared to 30 years old subjects [8].

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

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 (EPA) 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, 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, CS, have been demonstrated to be associated with cutaneous pathologies.

It is worth mentioning, at this point, the epidemiological work by Xu et al. (2011) where 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 were analyzed. 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 found. 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 for 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 starts its course 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 stratum corneum, 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 NOx) [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_2 was positively associated with eczema in children exposed to traffic-related air pollution [19]. In a more recent work, PM10 and NO_2 exposure during the first trimester of pregnancy was associated with the development of infantile AD [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 [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 [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 1/3 have smoked in the past [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 cigarette smoke and AD in non-smokers.

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 [24], CS [25], or O_3 [26] can activate the aryl hydrocarbon receptor (AhR), and this can further activate Th17 cells [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 [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 [28].

Interestingly, CS has been also connected with the development of acne [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 [29]. Indeed, the ability of PM, CS, and even O_3 to

increase 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) [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_3 exposure [31-33] (GV unpublished data).

In addition to the aforementioned skin conditions, environmental changes, due to the 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 [34]. Exposure to PM has been also implicated in the development, persistence, and exacerbation of other cutaneous conditions atopic dermatitis, acne, psoriasis, skin aging, androgenetic alopecia, and skin cancer [35].

In conclusion, ambient air pollutants, such as PM, CS, or $O_{3,}$, seem to be involved in the pathogenesis of inflammatory skin diseases (e.g., AD, acne, and psoriasis) *via* a common denominator through enhancing oxidative stress and pro-inflammatory mediators (OxInflammation phenomena) [36].

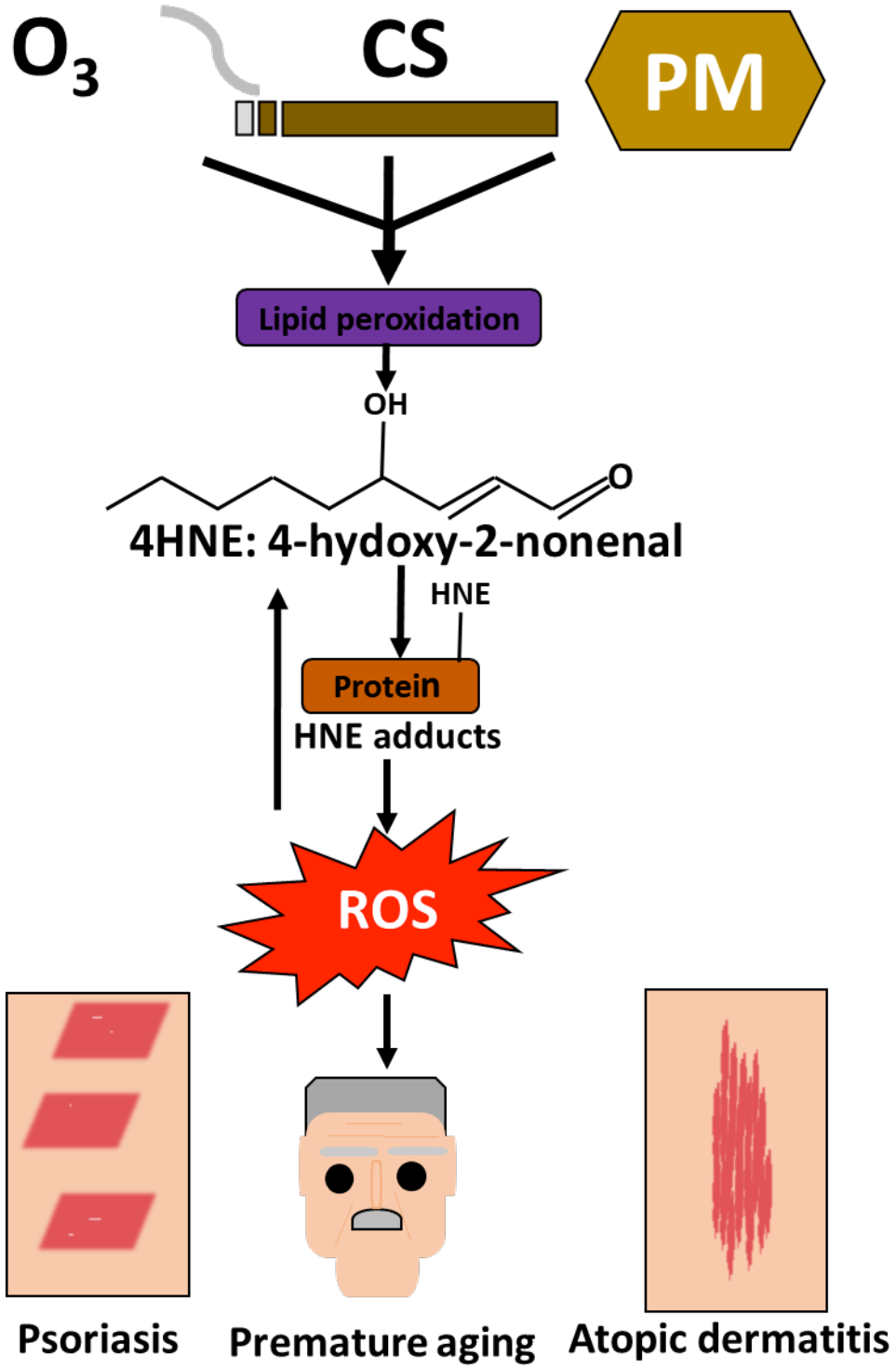

Figure 1. Consequences of 4HNE production in response to pollutants. Exposure of the skin to ozone (O_3), cigarette smoke (CS), and particulate matter (PM) induces lipid peroxidation and production of 4-hydroxynonenal (4HNE). This product of lipid peroxidation can form covalent bonds with the histidine, 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 atopic dermatitis.

MECHANISMS INVOLVED IN POLLUTION AFFECT CUTANEOUS TISSUES

ATMO$_3$SPHERIC 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_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 layers through the generation of a cascade of ozonation products. Although it is not a radical species *per se*, O_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, non-radical species including aldehydes. Due to its high reactivity and chemical and physical properties of low aqueous solubility within the skin, O_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 stratum corneum [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 O_3 can generate several secondary messengers able to trigger signaling cascades across the different layers of skin, leading to pro-oxidative and inflammatory processes [38-40].

For instance, in a recent study, it has been shown that ozone exposure for was able to increase levels of 4HNE in human skin, and this correlated with increased proinflammatory markers such as COX2 and NFκB [41]. Interestingly, the levels of 4HNE followed a clear gradient pattern, with high levels in the upper epidermis and lower levels in the dermis, suggesting that the effect of O_3 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 O_3 on cutaneous tissues is a consequences of its reaction with the lipids present in the stratum corneum.

This concept was first advanced by Pryor et al. [42] in relation to the respiratory tract, suggesting that exposure of non-cellular constituents of surface epithelial cells to O_3 is capable of generating potentially toxic peroxidation products. Extrapolation of this concept to cutaneous

tissues suggests that O_3 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 O_3 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 O_3 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 cell-specific, tightly regulated NAD(P)H-oxidase systems for generating oxidants, and further amplifying the oxidative stress damage [41].

As of today, the potential overall mechanism by which O_3 is able to affect skin has been described. It is generally understood that the toxic effects of O_3, 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 vitamin E and C. O_3 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 bioreactive species. Our lab together with other recent works, have provided some evidence that these bioactive compounds are likely to penetrate the underlying cutaneous tissues, as demonstrated by the presence of several pro-inflammatory 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 stratum corneum barrier, and diffusion of bioreactive products from this tissue into the viable layers of the epidermis, may represent a contribution to the development/exacerbation of skin disorders associated with O_3 exposure. Indeed, once these "mediators" are able to reach live cells (keratinocytes, fibroblasts, etc.) they can induce a cellular defensive and 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.

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 pro-oxidants [43], with more than 10^{14} low molecular weight carbon- and oxygen-centered radicals per puff present in gas-phase smoke [44]. 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 (TPM) than mainstream CS [45]. 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 [45].

As mentioned above, the toxic effect of cigarette smoke (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 [46]. Epidemiological studies strongly correlated CS to premature skin aging [47-49]. 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 grayskin (smoker’s melanosis) and deep wrinkles (smoker’s wrinkle) [50]. Indeed, wrinkle formation is a typical feature associated with tobacco smoking [51]. CS is able to affect skin aging by activating MMPs in the connective tissues [52]. 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 [53]. The mechanisms involved in CS-induced skin aging remain unresolved, although it is believed that activation of the arylhydrocarbon receptor (AhR) signaling pathway contributes to this effect.

CS contains water-insoluble polycyclic aromatic hydrocarbons (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 [54, 55]. Once activated, AhR dissociates from some of the proteins in the inactive complex and translocates to the nucleus, where it dimerizes with Arnt [56]. The AhR/Arnt heterodimer activates the transcription of xenobiotic-metabolizing genes [57, 58]; some of which encode proteins involved in growth control, cytokines, nuclear transcription, and regulators of extracellular matrix proteolysis [59, 60].

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 [61].

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 [28]. 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_2O_2 levels and mitochondrial superoxide production [31]. We also observed that CS exposure in keratinocytes increases levels of HNE and acrolein adducts [31]. 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 [62]. Moreover, the increased levels of superoxide anion or H_2O_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 4HNE. Since increased oxidative stress in the skin has been associated with premature aging [63-65], the ability of CS to induce oxidative damage can likely contribute to premature aging.

Mechanisms involved beyond PM-induced skin damage

Particulate matter is a complex, heterogenous 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, 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 UFPs. Coarse particles have a diameter of 2.5 to 10 μm (PM_{10}) and can be generated by farming, mining, and construction [66]. Fine particles have a diameter of 2.5 μm or less ($PM_{2.5}$) and can be generated by power plants, oil refinishes, fuel combustion, cars, and wildfires [66]. There are also ultrafine particles (UFPs) with diameters less than 0.1 μm or 100 nm that can be generated by diesel and gasoline fuel combustion, cars, aircrafts, and ships [66-68]. 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 [69]. Inhalation of $PM_{2.5}$ results in increased plaque deposits in arteries, promoting development of atherosclerosis as well as increased risk of heart attacks [70, 71].

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 [1]. Exposure to PM is associated with progression of atopic dermatitis in children [72], and improving air quality resulted in decreased prevalence and severity of atopic dermatitis [73].

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. Polycyclic aromatic hydrocarbons (PAHs) are components of UFPs that can be absorbed through the skin and eventually damage the mitochondria, resulting in intracellular ROS production [74]. These damaged mitochondria produce superoxide anions, which can be converted into H_2O_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 AP-1 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 AP-1 and NFκB. The consequences of oxidative stress results 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 4HNE, and promotes DNA damage in *ex vivo* human biopsies [75]. The consequences of increased oxidative stress in response to PM exposure results in the exacerbation of preexisting skin diseases and premature skin aging [1].

4HNE: the trigger for pollution-induced skin OxInflammation

4HNE derives from the oxidation of ω-6 PUFAs, essentially arachidonic and linoleic acid, i.e. the two most represented fatty acids in biomembranes. 4HNE is an unusual compound containing three functional groups that in many cases act in concert, explaining its high reactivity (Fig. 2). There is, first of all, a conjugated system consisting of a C=C double bond and a C=O carbonyl group in 4HNE. The hydroxyl group at carbon four contributes to reactivity both by polarizing the C=C bond and by facilitating internal cyclisation reactions, such as thio-acetal formation [76, 77].

4HNE is an amphiphilic molecule; in fact, it is water soluble and also has strong lipophilic properties. Consequently, 4HNE tends to concentrate in biomembranes, where phospholipids, like phosphatidylethanolamine, and proteins, such as transporters, ion channels, and receptors, quickly

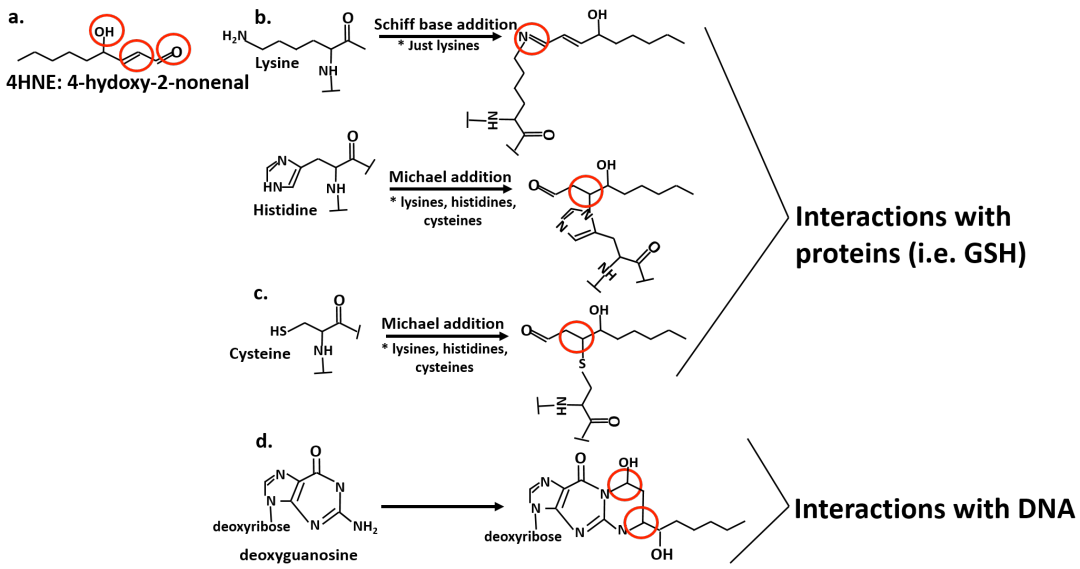

Figure 2. Schematic representation of adduct formation by 4HNE. (a) 4-hydroxynonenal (4HNE) contains three functional groups: a C=C double bond, a C=O carbonyl group, and a hydroxyl group at carbon four. All of these functional groups contribute to its reactivity. (b, c) 4HNE can form HNE protein adducts through Schiff base addition to lysines and Michael addition to lysines, histidines, and cysteines. Specifically, 4HNE can form adducts with glutathione through cysteines present in this protein. (d) 4HNE can also damage DNA by forming adducts with nitrogenous bases (i.e. deoxyguanosine). In red, we have indicated where bonds form between HNE and its partner.

react with 4HNE. 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 (Fig. 2) [78]. Because of its electrophilic nature, 4HNE can form adducts with cellular protein nucleophiles. Indeed, the reactivity of 4HNE explains its potential involvement in the modulation of enzyme activity, signal transduction, and gene expression [76, 77].

Besides being a product of oxidative stress, 4HNE is also an efficient cell signalling 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 bulk of literature indicates that 4HNE, 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, 4HNE may modulate redox-sensitive transcription factors such as nuclear factor-kappa B (NFκB), activator protein-1 (AP-1), and nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Moreover, its proven interaction with a variety of enzymes and kinases variously involved in cell signalling, strongly support its important role in pathophysiology as a cell signalling messenger [76, 77].

4HNE: METABOLISM, TOXICITY, AND PROTEIN ADDUCTS

Once formed, under physiological conditions, 4HNE 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 conjugation of GSH to 4HNE via Michael addition at the C-3 carbon, thereby preventing further nucleophilic addition to this toxic compound. Aldehyde dehydrogenase catalyzes the oxidation of 4HNE to the innocuous 4-hydroxy-2-nonenoic acid (HNA), while alcohol dehydrogenase catalyzes reduction of the terminal aldehyde to its alcohol, yielding the unreactive metabolite 1,4-dihydroxy-2-nonene (DHN). Another enzyme involved in the metabolism of 4HNE 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 4HNE, leading to DHN–GSH [76, 77].

The half-life of 4HNE has been studied in several cell types, in subcellular organelles, and even in whole organisms. Liver tissue generally has the highest capacity to metabolize 4HNE, while in other cells, the metabolism of 4HNE is not so fast, but still very efficient [76, 77]. Usually, 4HNE, even at very high lipid peroxidation rates, cannot accumulate in an unlimited manner. However, compared with other oxidants, such as most types of ROS, 4HNE 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 4HNE molecules, 4HNE can escape detoxifying processes and migrate from the site of origin to other intracellular sites, reacting rapidly with biological macromolecules, especially proteins to form 4HNE protein adducts (PAs) [79].

4HNE protein adducts are physiological constituents of mammalian organisms. They are easily detectable in peripheral blood, where they primarily involve albumin, transferrin, and immunoglobulins, but also proteins related to blood coagulation, lipid transport, blood pressure regulation, and protease inhibition [76, 77]. Nevertheless, as proteins play an important role in the normal structure and function of cells, oxidative modifications promoted by increased 4HNE levels, as in altered redox homeostasis conditions, 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, 4HNE can modify mitochondrial proteins such as cytochrome c, impairing mitochondrial metabolism [80]. 4HNE PAs also contribute to the pool of damaged enzymes, which increases in levels during aging and in several pathological states [81].

4HNE can activate a variety of signal transduction pathways including the Erk pathway, p38MAPK, JNK pathway, and EGFR pathway [82]. 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, 4HNE can induce Nrf2 activation by modifying its inhibitor Keap1, releasing Nrf2 for nuclear export [83]. Moreover, it can enhance the DNA binding activity of AP1 and negatively and positively regulate NFκB [82, 84]). Because of these findings, it is believed that 4HNE can be causally involved in many of the pathophysiological effects associated with oxidative stress in cells and tissues [85], especially for the skin due to its rich concentration of omega-6 fatty acids.

CONCLUSION

Indeed, the presence of 4HNE adducts in skin after pollution exposure has been well documented and in several cases linked to skin aging, making this marker as a possible common mediators of skin oxidative damage. For instance, 4HNE levels were increased after O_3 exposure in both 2D and 3D models [86, 87] and confirmed also in human skin biopsies after 5 days of O_3 exposure [41]. These results are in line with previous animal work, wherein hairless mice exposed to O_3 exhibited a clear increase of 4HNE PAs in the epidermis [37]. Interestingly, cutaneous 4HNE levels were also significantly higher after O_3 exposure in old animals, compared to young animals, in a wound healing study, suggesting their role in delaying cutaneous wound closure in aged mice

[63], possibly via the aberrant activation of MMPs [64]. O_3 is not the only pollutant that induces the formation of cutaneous 4HNE in the skin; indeed, similar effects have been observed after both CS and PM exposure. Our group was able to demonstrate the formation of 4HNE PAs in both keratinocytes and reconstituted human epidermis tissues (RHE) after CS and PM exposure [75, 88]. The formation of 4HNE PAs, as mentioned before, leads to the covalent modification of proteins, which can be subsequently ubiquitinated and degraded. Therefore, a consequence of 4HNE adducts formation is the loss of important cellular proteins, such as SRB1 [31], which we observed in both keratinocytes and sebocytes [30, 31]. In a very recent work by Verdin *et al*. (2019), the ability of UFPs to increase 4HNE levels in RHEs was also observed, suggesting a role of 4HNE in regulating skin differentiation and cornification [89]. Several studies have demonstrated a role for 4HNE in skin aging [90, 91] and in skin colour [92]. In addition, 4HNE PAs have been shown photo-damaged skin elastosis [93].

Therefore, it is possible that the induction of 4HNE by pollution in the skin could accelerate skin aging and also exacerbate existing skin pathologies. For instance, 4HNE levels have been also detected in skin samples from psoriatic patients as well as in cases of atopic dermatitis [94, 95]. Being able to prevent cutaneous 4HNE formation could be a possible innovative cosmeceutical approach for future topical applications.

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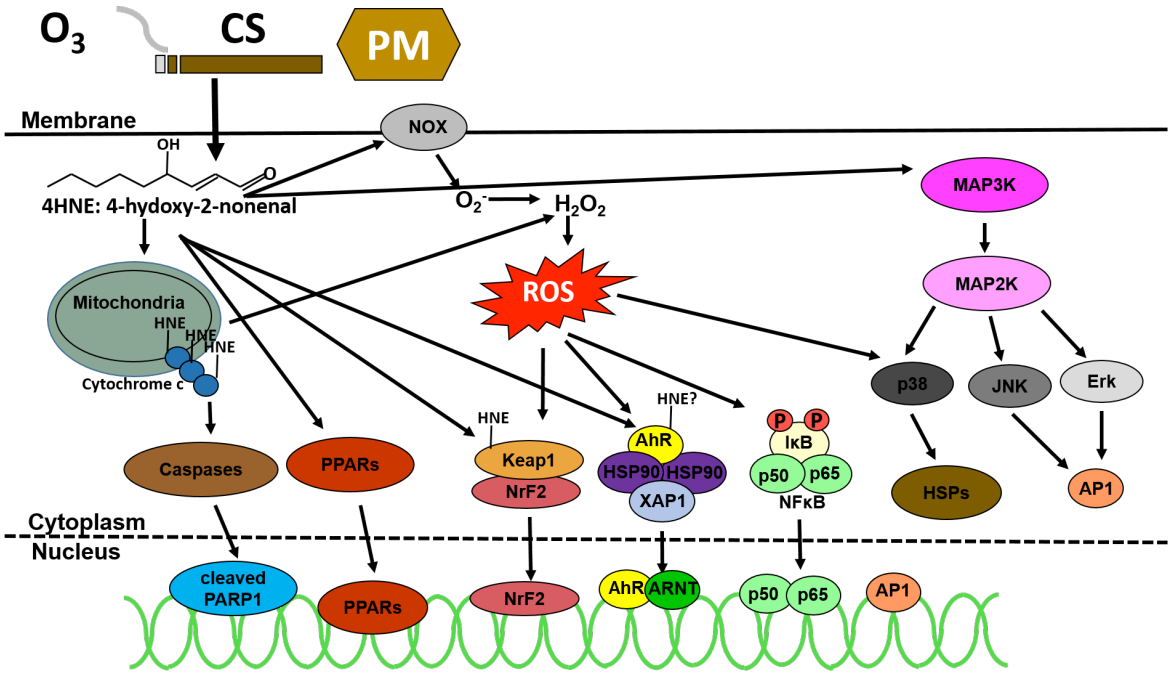

Figure 3. 4HNE as pollutant-induced signaling mediator. Exposure of the skin to ozone (O_3), cigarette smoke (CS), and particulate matter (PM) induces lipid peroxidation and subsequent production of 4-hydroxynonenal (4HNE), resulting in the Michael addition of HNE to protein products such as cytochrome c, which results in ROS production via mitochondria, and Keap1, which releases NrF2 from sequestration. In addition, 4HNE activates NAPDH oxidase, again resulting in the generation of ROS and oxidative stress, which can activate NFκB and AhR. 4HNE 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.

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