EXTENDED ABSTRACT

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Cutaneous oxidative stress induced by pollution (particulate matter) and its aggravation by environmental ultraviolet radiation

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Abstract:

Atmospheric pollution is a serious health concern and particulate matter (PM) is considered as particularly deleterious. In fact, ultrafine particles contain toxic compounds such as poly aromatic hydrocarbons (PAH) adsorbed at their surface. Moreover, they can reach blood circulation and be distributed in the whole body, PAH plasma concentration reaching the nanomolar range. Contamination of deep skin, either by penetration or by systemic exposure is probable. Some PAH are phototoxic: sunlight and pollution might thus synergistically compromise skin health. At concentrations in the nanomolar range, some PAH (benzopyrene or indenopyrene) displayed a strong phototoxicity on keratinocytes under exposure to daily UV (300-400 nm) or to UVA1 (340-400 nm). PAH-induced photo-oxidative stress could impair mitochondrial function (membrane polarization and ATP production) and impacted endogenous glutathione homeostasis. Interestingly, among genes controlling glutathione metabolism, SLC7A11 was particularly overexpressed (at gene and protein levels). This suggests that regeneration of GSH might be of huge importance to ensure protection against "photo-pollution" stress. As proof, pretreatment of cells by buthionine sulfoximine BSO, an inhibitor of GSH biosynthesis, significantly increased PAH-induced phototoxicity. Our results highlight that pollutants could aggravate skin photodamage: specific photoprotection strategies for skin care in polluted area are thus necessary.

Introduction:

Skin is exposed to several environmental stresses of differing nature and intensity and interest in pollution has recently increased because of its adverse impact on health. Recent clinical data suggested that pollution aggravated atopic dermatitis and skin aging (Krutmann et al., 2014; Mancebo et al., 2015). Pollution involves a large range of chemicals, with particulate matter (PM) being probably the most harmful (Drakaki et al., 2014; Kim et al., 2016). Particles from wood/coal burning and diesel exhaust particles are small ($PM_{2.5}$: median size 2.5 µm) and include ultrafine particles with diameters under 100 nm. $PM_{2.5}$ is probably a major harmful pollution component because this class contains toxic chemicals, particularly PAH (Polycyclic Aromatic Hydrocarbons). Transcutaneous penetration of PM through healthy skin may be limited due to poor bioavailability of particles larger than 20 nm. PAH could also reach deeper skin strata via the systemic route as UFP deposited in pulmonary alveoli translocate across the epithelial barrier into pulmonary capillaries. Traces of UFP or PAH could thus be released from circulating blood into the dermis and deep epidermis at concentrations possibly comparable to those in serum (nanomolar range). Moreover, the well-known photoreactivity of some PAH upon UVA exposure could enhance the deleterious impact on skin. In fact, PAH phototoxicity, particularly BaP, has been investigated *in vitro* for decades. *In vivo*, small amounts of topical BaP were photocarcinogenic in mice. Photoactivation of PAH traces may thus generate a detrimental chronic stress in skin and related mechanisms require further investigations.

Materials and methods:

Benzo[a]pyrene (B1760), Indeno[1.2.3-cd]pyrene and Buthionine Sulfoximine (BSO) were purchased from Sigma-Aldrich. Diesel Particulate Matter

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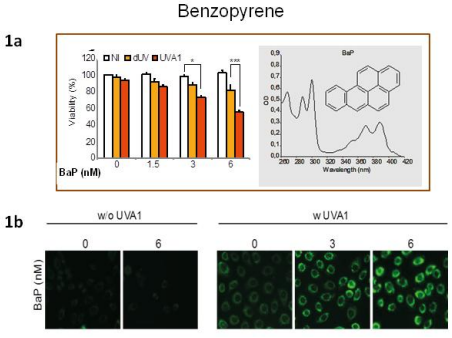
(PM) and Diesel Particulate Matter Extract (PME) were supplied by the National Institute of Standards and Technology (NIST): Standard Reference Material 1650b (PM), and 1975 (dichloromethane extract of diesel particulate matter). Normal human epidermal keratinocytes were obtained after dissection and treatment of foreskin biopsies were cultured in KGM-gold medium (Lonza, Walkersville, MD, USA). The light source was a solar UV simulator (Oriel, Stratford, CT, USA) equipped with a 1000 W xenon short arc lamp and a dichroic mirror. dUV and UVA1 were obtained using the WG320 filter (Schott, Clichy, France) and the UVA1 filter (Monaderm, Monaco), respectively.

Results and discussion:

Combined exposure to Daily UV (UVB+UVA) or UVA1 and particulate matter (PM) or PM extract (PME) triggered a comparable phototoxic impact.
The Daily UV (d-UV) wavelength range (300-400 nm) includes attenuated UVB radiation and the entire UVA band (UVA1+UVA2) whereas UVA1 radiation ranges 350-400 nm. The phototoxicity induced by particulate matter PM and particulate matter extract PME in keratinocytes exposed to both UV ranges was compared at the same UV dose as assessed by spectroradiometry (7.5 J/cm^2). Surprisingly, cell survival was similar in both UV ranges despite the higher absorption of PME in the UVB and short-wave UVA ranges. Thus, in our experimental conditions, UVA1 seemed to mostly contribute to this phototoxic process. Among several PAH, Benzopyrene BaP and Indenopyrene IcdP behaved as nanomolar UVA1 photosensitizers in our experimental conditions (figure 1a, c).
BaP and IcdP induced photo-oxidative stress involving mitochondria.



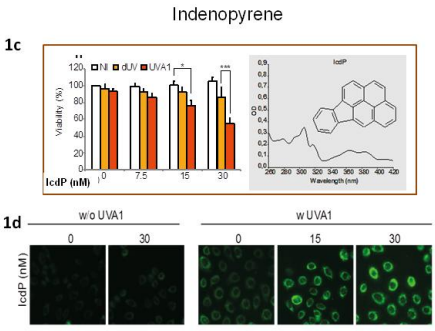


Figure 1. Viability of keratinocytes exposed to BaP and IcdP and UV and induction of photo-oxidative stress. Viability of cells exposed to Benzo(a)pyrene (BaP) (1a) or Indeno[1,2,3-cd]pyrene (IcdP) (1c) in the dark (NI) or exposed to 7.5 J/cm^2 of d-UV or UVA1. Mean of three independent experiments ± SD. *p<0.5, **p<0.01 and ***p<0.001 *versus* Ctrl [two-way analysis of variance (ANOVA) with Dunnett's test]. Representative aspect of keratinocytes stained with DHR-123 after exposure to BaP (1b) or IcdP (1d) and UVA1.

It has been reported that the photoreactivity of PAH produced various reactive oxygen species and that UFP could enter and damage mitochondria following cellular uptake. The strong fluorescence emitted by the DHR-123 probe immediately after irradiation demonstrated that BaP and IcdP enhanced UVA1-induced oxidative stress inside cells (figure 1b,d). Moreover, after exposure to IcdP and UVA1, membrane depolarization (MMD) and superoxide anion production were detected in keratinocytes mitochondria whereas UVA1 alone or PAH treatment in the dark had no effect. Curiously, BaP+UVA1 had no significant impact on MMD or superoxide generation, whereas BaP was more effective than IcdP in impairing ATP production. These data show that low concentrations of both PAHs catalysed intracellular ROS production under UVA1 exposure and compromise mitochondrial function.
BaP and IcdP + UVA1 triggered a GSH metabolic response

Reduced glutathione (GSH) plays an essential role in intracellular redox homeostasis, particularly within mitochondria. Moreover, GSH is involved in the PAH detoxification process. Impact of PAH+UVA1 on GSH status was investigated in cultured keratinocytes. GSH levels were significantly but mildly decreased at 5 hours post UVA1 + BaP or IcdP exposure, but recovered initial levels by 18 hours (figure 2a). To further evaluate the involvement of GSH in cell protection, keratinocytes were pre-treated with buthionine sulfoximine (BSO), a GSH biosynthesis inhibitor. BSO pre-treatment significantly decreased keratinocyte viability following exposure to BaP or IcdP (Figure 2b). No impact on viability was observed after exposure to UVA1 alone. This result

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highlighted the likely role of GSH protection against the phototoxic stress produced by PAH+UVA1 within cells. To understand how cells adapted their redox status, the expression of genes involved in GSH homeostasis was studied. Catalytic and modulatory Glutamyl-Cysteine Ligase (GCLc and GCLm, respectively) control GSH neo-synthesis, Glutathione Reductase (GSR) regenerates oxidized GSH and SLC7A11 encodes an antiporter that controls the intracellular cystine supply.

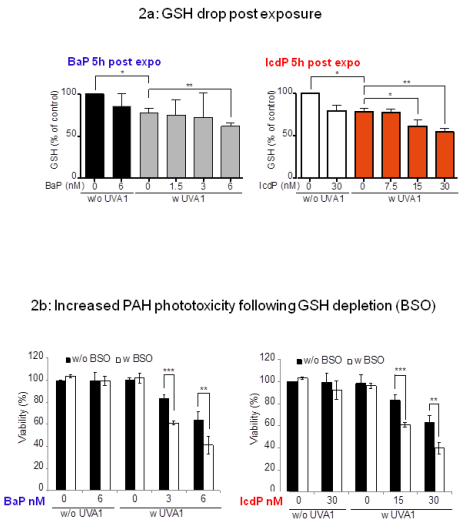

Figure 2. GSH concentration in cells after exposure to BaP or IcdP and UVA1. Effect of BSO pretreatment on phototoxicity of BaP and IcdP. (2a) Glutathione (GSH) quantification by LC/MS 5h after treatment in keratinocytes exposed to BaP or IcdP + UVA1 (7.5 J/cm2). (2b) Keratinocytes were pre-treated with DMSO (black bar) or BSO (white bar) for 24h and with BaP or IcdP with or without UVA1 (7.5 J/cm2). Cell survival was assessed 24 hours post irradiation by the MTT assay. Mean of three independent experiments ± SD. *p<0.5, **p<0.01 and ***p<0.001 *versus* Ctrl [two-way analysis of variance (ANOVA) with Tukey's test].

Figures 3a to 3d show that SLC7A11 gene was strongly induced 5 hours post UVA1 exposure, in presence of BaP and IcdP. SLC7A11 was also overexpressed at protein level. Expression of GCLc was stimulated to a lesser extent, whereas expression of GCLm and GSR appeared unaffected. This study aimed to investigate *in vitro* the cutaneous impact of pollution combined with exposure to sunlight. In our experiments, we used nanomoles of PAH to simulate a potential contamination of deep skin by some pollutants. We focused on UVA1 radiation, as it represents around 80% of daily UV and reaches the dermal-epidermal junction with ease. Moreover, due to its energetic properties, UVA1 radiation is relatively stable at different times of year and at different latitudes whereas UVB/UVA ratios vary. Our results showed that traces of PAH combined with UVA1 exposure produced a high cellular stress. It is probable that PAH is present in skin *in vivo* in low nanomolar concentrations either due to penetration of UFP into damaged skin or systemic distribution in the circulation. UVA1 radiation diffuses deep into the skin and its irradiance remains high over most of the day. Therefore, a chronic photo-metabolic stress may occur in the skin of individuals living in polluted environments, probably promoting cutaneous dysfunctions and accelerating the aging process.

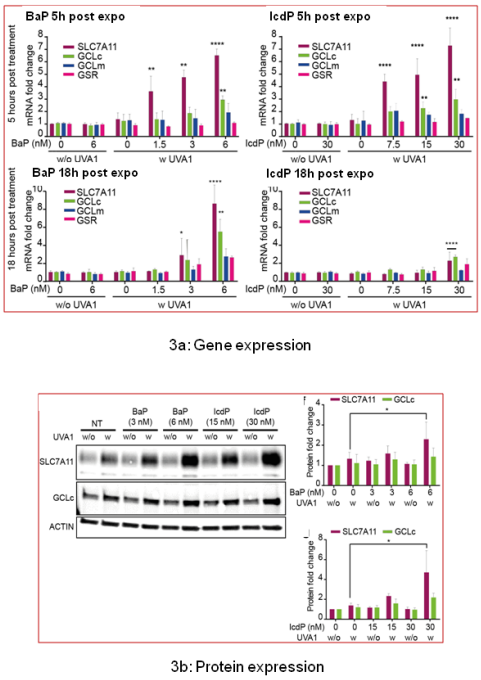

Figure 3. Expression of genes or proteins associated to GSH metabolism after exposure to BaP or IcdP and UVA1. (3a) Expression of genes involved in GSH homeostasis (SLC7A11, GCLc, GCLm, GSR) in response to treatment with BaP or IcdP in keratinocytes with or without UVA1 irradiation (7.5 J/cm2) analyzed by quantitative RT-PCR 5 hours or 18 hours post treatment. (3b) Expression of SLC7A11 and GCLc protein quantified by Western Blot 13 hours post treatment. Mean of three independent experiments ± SD. *p<0.5, **p<0.01 and ***p<0.001 versus Ctrl [two-way analysis of variance (ANOVA) with Bonferonni test for genes expression quantification and with Tukey test for protein quantification].

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