Schisandra chinensis combats pollution-induced stress

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Abstract

The human skin, and mainly the upper layer of the epidermis, plays the role of a barrier, but is also one of the first and major targets of air pollutants, pollutants contributing to wrinkle and dark sports occurrence through the redox imbalance. A possible approach to attack ROS-mediated disorders for both preventive and treatment means is based on the use of substances, which can be found in plants as secondary metabolites, lignans being a promise candidate. The present study was aimed to better understand the cellular mechanisms beyond the oxidative changes induced by urban pollution (Urban dust 1649b, NIST) and the effect of *Schisandra chinensis (S. chinensis)* extract in reconstructed human epidermis, by a transcriptomic approach and secondly through the evaluation of Nrf2, AhR, NF-kB, and DJ-1pathways using an *in vitro* model. Finally, we evaluated the effect of *S. chinensis* on skin hydration, homogeneity, radiance and luminosity in Chengdu (China). Urban dust (80µg.mL^{-1}) was able to activate the cytoplasmic expression of NF-kB and AhR when compared to control. *S.chinensis* extract attenuated the urban dust-induced oxidative stress, the protective mechanism being associated, at least in part, with the modulation of the Nrf2 and AhR pathways and the activation of DJ-1. *S. chinensis* extract, named Urbalys® protects from prolonged pollution aggression since it improves hydration, protects skin homogeneity, increases skin radiance and attenuates skin spot intensity after 21 days of pollution exposition.

Atmospheric pollution, which contains a quantity of microscopic suspended particulate matter (PM) carrying various toxic chemical molecules, including polycyclic aromatic hydrocarbons (PAHs), has to be considered nowadays as one of the main characteristics of areas where, worldwide, human population density is at a high level. It affects many rural, industrial, and urban sites[1] and has been recognised as the most important environmental health issue in the world. However, few studies concern the impact of air pollution and PM on skin integrity, even if it has been shown that they are significantly associated with weakened barrier function, oxidative stress, skin diseases, and skin ageing.[2] PM penetrate skin either through hair follicles or transdermally, and exert its detrimental effects through the generation of reactive oxygen species (ROS), which contributes to extrinsic skin ageing. ROS activate the mitogen-activated protein kinase (MAPK) signaling pathway inducing metalloproteinases (MMPs) production which are closely related to inflammatory skin diseases and skin ageing (Fig 1).[1]

PAHs and PM are also well established to activate the aryl hydrocarbon receptor (AhR), a pollutant sensor that constitutes the starting point of the detoxification mechanism, by upregulating the transcription of responsive genes, such as cytochrome P450 superfamily. However, these activations generate ROS. Endogenous defense mechanisms including a fundamental biochemical pathway nuclear factor erythroid 2–related factor 2 (Nrf2) is activated in order to fight the deleterious effects of all pollutants on skin. It is able to help eliminate and inactivate exogenous toxic agents by fundamental biological pathways closely interconnected. Nrf2 is constitutively expressed in the cytoplasm, and its accumulation and activation in the nucleus are favoured in oxidative injury. Additionally, Nrf2 is stabilised by the Parkinson's-associated protein, (DJ-1), a multifunctional protein expressed in almost all tissues involved in various physiological processes such as transcriptional regulation, anti-oxidative stress reaction, mitochondrial regulation, and signal transduction (Fig 1).[3] More precisely, DJ-1 promotes Nrf2 binding to antioxidant response elements by which Nrf2 can regulate the expression of several endogenous antioxidative enzymes and reduce ROS production to protect mitochondria and can also respond to oxidative stress. Under oxidative stress, DJ-1 plays critical antioxidant defence roles by several molecular processes. Additionally, it protects mitochondria by directly maintaining mitochondrial complex I activity and translocating into mitochondria as an endogenous antioxidant.[4]

A possible approach to attack ROS-mediated disorders for both preventive and treatment means is based on the use of substances which can be found in plants as secondary metabolites. Various phytochemicals and herbal extracts exert their antioxidant properties by activating the Nrf2 system. *Schisandra chinensis* is a traditional Chinese herbal medicine that has been used for the treatment in Asia for thousands of years. The lignans as the main active ingredients in *S. chinensis* have various pharmacological effects such as anti-oxidative, anti-inflammatory, antitumor, and hepatoprotective activities. Schisandra extract also seems to inhibit IκB activation, thereby suppressing the production of TNF-α, IL-6.5 These findings led us to postulate that *S. chinensis* lignans might protect skin cell functions against urban pollution and could be used as cosmetic agents. Thus, the objective of this study was firstly to identify the effects of urban dust by a transcriptomic approach on reconstructed human epidermis and then the effect of urban dust and *S. chinensis* extract on the expression of genes involved in response on cellular protection mechanisms, namely the detoxification pathways. Secondly, we examined the effect of active extract from *S. chinensis* on the protection of human keratinocytes damages caused by pollution

through the evaluation of Nrf2 and AhR pathways, NF-kB, and DJ-1. Finally, we evaluated the effect of *S. chinensis* on skin hydration, homogeneity, radiance and luminosity in Chengdu (China).

Materials and methods

The urban dust chosen was SRM1649b from Sigma including PAHs and nitro-PAHs, polychlorobiphenyls, chlorinated pesticides, decabromodiphenyl ether, polychlorinated dibenzo-p-dioxins and dibenzofurans, inorganic constituents, heavy metals, and particulate matter (PM2.5 and PM10).

Transcriptomic analyses

The transcriptomic approach was conducted on a 3D model of reconstructed human epidermis (RHEs) and consisted to measure by qRT-PCR the effects of *S. chinensis* extract on the expression of 93 genes involved in response and cellular protection mechanisms against pollution, namely the detoxification pathways, the inflammation and the antioxidant defence.

Effect of extract from *S.chinensis* on the protection of human keratinocytes (NHEK) damages caused by pollution

After the cytotoxicity evaluation for determination of cell viability using WST1 assay, Normal Human Epidermal Keratinocytes (NHEK) cells were cultured overnight at a 5000 cells/well of density in a 96 well plate, at 37°C, 5% CO_2. The cells were treated then 24 hours with the *S. chinensis* extract 0.0.1% or control, then exposed during 6h to urban dust (80 µg.ml^{-1}) and *S. chinensis* extract. The cells were fixed with formalin and the expression of AhR, Nrf2, DJ-1, and NF-kB were detected by immunofluorescence. After a step of permeabilisation/saturation, staining of the treated cells with antibodies (anti-AhR polyclonal, anti-Nrf2 polyclonal, anti-DJ-1 polyclonal and anti-NFkB polyclonal) was performed overnight at 4°C.The secondary antibody AlexaFluor 488 tagged goat antibody was applied 1h at RT. Fluorescent labeling was imaged and quantified by automated microscopy (Array Scan Cellomics TM). The fluorescence was quantified by the bioapplication Compartmental Analysis.

Clinical study

The study was performed by comparison before and after hemi-face application of *S. chinensis* extract 1% *vs* placebo, twice a day. Thirty-one women were involved from 21 to 51 years old, with dilated pores or oily or dull skin or spot and with a wash-out of 15 days with placebo. Study was performed during 21 days where subjects have to be at least 4 hours a day in a polluted urban area. The different parameters analysed at D0, D7, D14, and D21 were: Clinical scorage and self-assessment, Skin hydration by Corneometer CM825, Radiance and luminosity by glossymeter GL200 and spectrocolorimetry CM2600d.

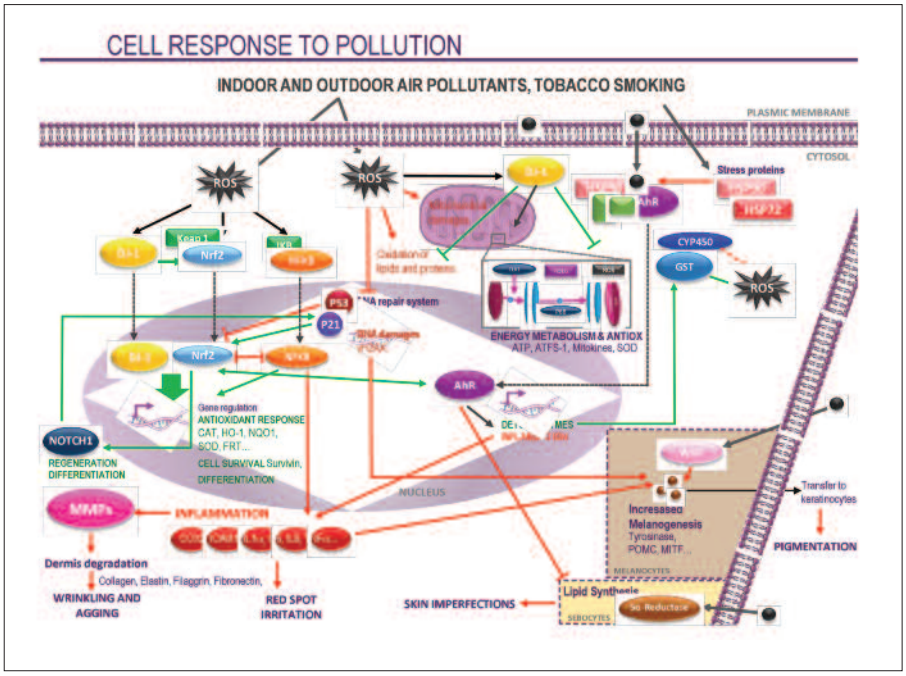

Figure 1: Signalling pathway responses to global pollution.
Air pollutants induce ROS generation and pro-inflammatory cytokines. Upon ligation by pollutants, the activated AhR translocates from the cytoplasm into the nucleus. This translocated AhR binds with ANRT, resulting in the activation of Cytochrome P450, family 1, member A1 (CYP1A1) transcription. ROS generated by CYP1A1 stimulates the production of TNF-α and IL-8. ROS also activates beneficial cellular responses, including Nuclear factor(erythroid-derived 2)-like 2 (Nrf2) activation. Under normal conditions, Nrf2 localizes in the cytoplasm where it interacts with the actin binding protein, Kelch-like ECH associating protein 1(Keap1), and is rapidly degraded by the ubiquitin-proteasome pathway. Signals from ROS target the Nrf2-Keap1 complex, dissociating Nrf2 from Keap1. The Parkinson's-associated protein, DJ-1, is indispensable for Nrf2 stabilisation, by affecting Nrf2association with its inhibitor Keap1. Stabilised Nrf2 translocates to the nuclei, binds to the antioxidant response element and thereby regulates the expression of a large battery ofgenes involved in the cellular antioxidant protection, including NADPH quinine oxyreductase(NQO-1), heme oxygenase-1 (HO-1), glutathione (GSH)... ROS can also activate gene transcription via transcription factors, such as NF-kB that can interact directly with specific DNA motifs on promoters of target genes. The transcriptions of several MMP family members are strongly regulated by NF-kB. Increased activities of NF-kB lead to collagen breakdown, the downregulation of type I procollagen, and upregulations of MMPs resulting in premature skin ageing.

Statistical analysis

Results were expressed as mean value ± SEM and represent the mean of triplicate determinations obtained in four separate experiments. ANOVA followed by Bonferroni post-hoc test were performed by SPSS Software (version 16.00 for Windows, US) and statistical significance was considered at $p < 0.05$.

Concerning the clinical study, descriptive statistics are expressed as mean (± standard-deviation). Evolutions between visits evaluated using analyses of variance in repeated measures.

Results

Transcriptomic results

Urban dust (80µg.mL-1) increases significantly the expression of CYP1A1 ($p< 0.001$), and CYP1B1 ($p< 0.001$), and ALDH3A1 ($p<0.001$). Other effects of urban dust include over-expression of metalloproteinases MMP-1 ($p< 0.01$) and MMP-9 ($p< 0.04$) and an increase in GPX ($p<0.006$) and NAD(P)H deshydrogenase ($p< 0.001$).

In presence of urban dust, *S. chinensis* extract (0.01%) activates over-expression of several genes involved in antioxidant response and in detoxification pathway, including FTL (+21%: $p< 0.03$), and GPX2 (+37%: $p< 0.007$). It also induces the over expression of SPRR1A (+20%: $p<0.02$) that is a gene coding for a cornifin-A, functioning as a cross-linked envelope precursor.

Effect of urban dust and co-treatment on NF-kB activation and cellular pathways in human keratinocytes

Urban dust significantly activated the cytoplasmic expression of NF-kB (+16%, $p<0.001$), when compared to control. Co-treatment of urban dust and *S. chinensis* extract decreased significantly it expression in cytoplasm (-8%, $p< 0.001$) and nucleus (-6%, $p<0.001$).

Urban dust induced an increase in Nrf2

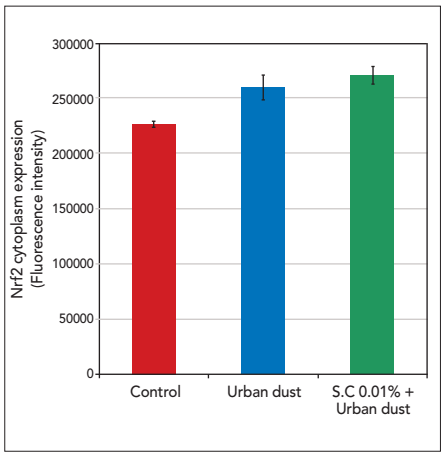

Figure 2: Nrf2 cytoplasm expression after urban dust exposure or co-treatment with urban dust and *Schisandra chinensis* (S.C.) extract.

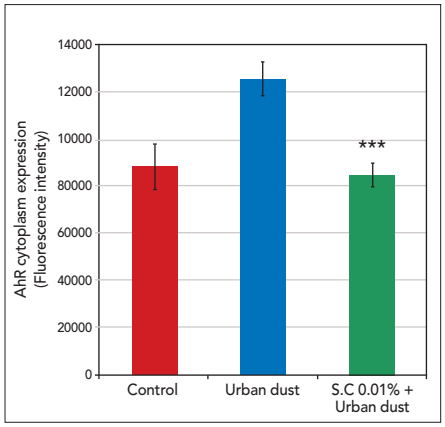

Figure 3: AhR cytoplasm expression after urban dust exposure or co-treatment with urban dust and *Schisandra chinensis* (S.C.) extract. *** p< 0.001 versus urban dust.

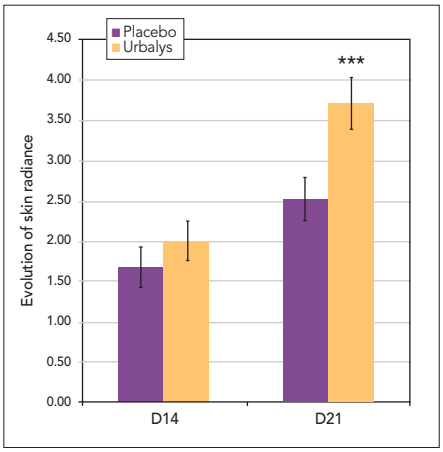

Figure 4: Evolution of skin radiance between *Schisandra chinensis* extract named Urbalys® 1% and placebo. Statistical significance *** p<0.001.

cytoplasmic (+15%, p<0.001) and nucleus (+28%, p<0.001) expression. The co-treatment demonstrated a higher cytoplasmic activity on Nrf2 (Fig 2) and a significant decrease in nucleus activity (-12%, p< 0.001), when compared with urban dust alone.

DJ-1 cytoplasmic (+91%, p<0.001) and nucleus (+47%, p<0.001) expression increased in NHEK exposed to urban dust, when compared to control. Co-treatment induced a higher cytoplasmic expression when compared to urban dust alone (+33%: p< 0.001). However, DJ-1 nucleus activity decreased after co-treatment.

Urban dust induced the expression of AhR in cells. In fact, the presence of AhR increases in both cytoplasm (+42%, p<0.001) and nucleus (+44%, p<0.001) (Fig 3). Co-treatment induced a return to basal values (control).

Clinical study

Skin hydration was measured via capacitance measurements. The measurable capacitance is mainly contributed by the stratum corneum water which is directly proportional to its water content. Treatment with *S. chinensis* induced a significant increase in skin hydration about 25% (p< 0.01) and 34% (p<0.001) respectively at D14 and D21 compared to placebo.

We observed that treatment with *S. chinensis* increases the radiance of the skin by 19% and 47% (p< 0.001) respectively at D14 and D21 (Fig 4).

Self-assessment of volunteers was also performed and, as shown in Figure 5. *S. chinensis* presents a satisfaction of most subjects allowing a less grey and less shiny skin, a luminous and unified complexion, an hydrated and smoother skin and less pores.

Discussion

Skin is inevitably exposed to environmental pollutants inducing oxidative stress which accelerate skin ageing and skin inflammation. The use of natural products in maintaining skin integrity and health against the deleterious effect of pollutants arouse great interest. *Schisandra chinensis* is a traditional Chinese herbal medicine that has been used for treatment in Asia for thousands of years. It is known to possess anti-oxidant and anti-inflammatory properties, through high concentrations of lignans.[6] Thus, the aim of our study was 1) to better understand the cellular mechanisms induced by pollution in keratinocytes and the effect of *S. chinensis* extract to fight protects the skin against ROS induced by urban dust, 2) to evaluated the effect of *S. chinensis* on skin hydration, homogeneity, radiance and luminosity in Chengdu (China) using an *in vivo* approach. We noted the protective effect of *S. chinensis* extract, which increased DJ-1 protein levels, Nrf2 expression and decreased AhR and NF-kB in cytoplasm even if the cells are under stress pollution. These results are in line with those of Lin et al.[7] who recently reported that Schisandrin B, an active ingredient extracted from *S. chinensis*, blocked the activation of NF-κB, and activated Nrf2, which resulted in the inhibition of inflammatory response. As previously mentioned, Nrf2 is an important transcription factor that plays a critical role in protecting cells from oxidative stress. Moreover, the increase of DJ-1 noted in our study after co-treatment of urban dust and *S. chinensis* extract is an important result, DJ-1 quenching the activity of ROS and protecting mitochondrial function.[8] It is also known that DJ-1 promotes Nrf2 binding to antioxidant response elements by which Nrf2 can regulate the expression of several endogenous antioxidative enzymes and reduce ROS production to protect mitochondria and can also respond to oxidative stress. We also noted that urban dust activates the AhR pathway (Fig 3), which is required for biotransformation of pollutants through the different phases of detoxification. It should however be noted that the first enzymes of the detoxification process can produce intermediate metabolites even more reactive than their exogenous precursors.[9] These results are in agreement with the fact that urban dust induces expression of genes of the phase I in our study. It is therefore of primary importance for the cell to achieve complete transformation and detoxification of such metabolites through the expression of genes coding for phase II enzymes. Our observations confirm that urban dust activates the drug metabolism response and especially the phase I dependent on the CYP450 family members as well as other enzymes such as MMP-1 and MMP-9. Activation of the MMP-1 gene has been shown to be critically linked to mitogen-activated protein kinase activation. Co-treatment of urban dust and *S. chinensis* extract induced a significant decreased of AhR expression (Fig 3), as compared to urban dust alone. Finally, the capability of *S. chinensis* extract to protect from prolonged pollution aggression was reflected by the fact that it improves hydration, protects skin homogeneity, increases skin radiance and luminosity and attenuates skin spot intensity after 21 days of pollution exposition.

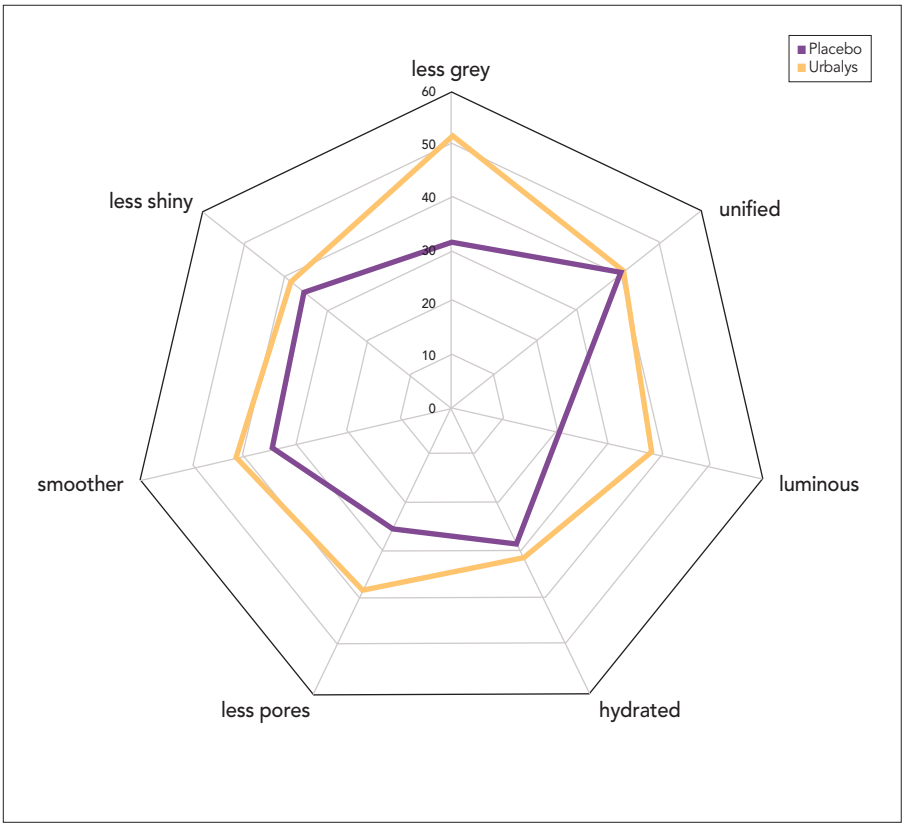

Figure 5: Self-assessment of volunteers: percentage of volunteers who fully agree with statements.

Conclusion

Using a translational approach and a standardised urban dust, we noted that treatment with *S. chinensis* extract attenuated the urban dust-induced oxidative stress and that the protective mechanism was associated, at least in part, with the modulation of the Nrf2 and AhR pathways and the activation of DJ-1. Moreover, the clinical study shows that application of *S. chinensis* extract, named Urbalys® during 21 days is sufficient to have beneficial effects on skin health. Urbalys counteracts deleterious effects of pollution by recovering the physiological balance of the skin. PC

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