

In vivo validation of the multicomponent powder (Vitachelox[®]) against the deposition of polluting ions

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Purpose: The purpose of this in vivo study is to evaluate the acute clinical application of a multicomponent powder (Vitachelox[®]), including three naturally occurring standardized extracts rich in polyphenols (grape seed extract, green tea extract, oak wood/bark extract), on healthy volunteers by measuring prevention of any metal deposition within the stratum corneum (SC) following a 6-h exposure period in a polluted environment.

Patients and methods: In this in vivo study, we evaluated the skin protective activity of the multicomponent powder formulated in a base emulsion compared to a relevant placebo cream. Using the tape stripping method, SC samples of face skin obtained from 30 healthy volunteers were compared following a 6-h exposure in a polluted area.

Results: No statistically significant variations on the amount of heavy metals were found in the samples of SC cells obtained from the hemi-faces treated with the multicomponent powder, with respect to baseline. On the contrary, a significantly higher concentration of heavy metals was found in the cells samples obtained from the hemi-faces treated with the placebo cream. In particular, an increased concentration of heavy metals superior to 100% were found for iron and zinc (+130.2% and +142.6%, respectively; $p < 0.001$).

Conclusion: This in vivo study validates and extends previous in vitro findings, indicating that the multicomponent powder allows the prevention of any metal deposition within the SC following exposure in a polluted environment. Our results suggest that the test product could play an effective role in counteracting skin damages induced by air pollution.

Keywords: air pollution, heavy metals, oxidative stress, polyphenols, green tea extract, oak wood extract, grape seed extract, antipollution, stratum corneum

Introduction

Exposure to indoor and outdoor air pollution represents a major public health concern.¹⁻³ Most studies focusing on air pollution effects on the lung and the cardiovascular system have shown that air pollutants are linked to heart diseases, respiratory infections, and lung cancer.⁴ More recently, epidemiological and mechanistic studies suggest that air pollution is also affecting skin integrity.⁵ 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. Continuous environmental exposure affects skin health, leading to aging, damages, and even skin diseases (such as erythema, edema, hyperplasia, contact dermatitis, atopic dermatitis, psoriasis, and carcinogenesis) if the environmental stressors exceed the defensive capacity of the skin.⁶ Major air pollutants with effects on the skin include the solar ultraviolet radiation, polycyclic aromatic hydrocarbons,

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volatile organic compounds, nitrogen oxides, particulate matter (PM), and cigarette smoke.⁷ PM is a complex mixture of liquid and/or solid inhalable particles suspended in gas derived from dust, soil, and traffic and industry emissions.³ Recent *in vitro* and epidemiological studies suggest that PM negatively affects human skin and influences the development and the exacerbation of preexistent skin diseases.^{8,9} Remarkable evidence shows that oxidative stress – namely increased reactive oxygen species (ROS) production – may be a common pathway in cellular responses of human skin to concentrated ambient particles exposure.⁸ In an *in vitro* study, Magnani et al⁸ showed that among the variety of compounds in air pollution, particularly transition metals participated in Fenton-like reaction and stimulated the cells to generate ROS.⁸ Besides the generation of free radicals (ROS and reactive nitrogen species), other mechanisms by which ambient air pollutants cause adverse effects on skin health include induction of inflammatory cascade and subsequent impairment of skin barrier, activation of the aryl hydrocarbon receptor, and alterations of skin microflora.¹⁰

In this light, external application of natural antioxidants may offer protection against the impacts of air pollution, favoring protective mechanisms of healthy skin that counteract oxidative stress. Among natural exogenous antioxidants, polyphenols are plant-based molecules that have antioxidant properties. It is also well known that polyphenols are effective metal chelators¹¹; the polyphenol–metal interactions (particularly with iron) could represent an antioxidant-causing mechanism.¹²

Besides their antioxidant properties, polyphenols also exert antiinflammatory and antimicrobial activities, which altogether make them promising tools in alleviating skin damages and preventing the development of various skin disorders.¹³

In a previous *in vitro* study,¹⁴ we evaluated the capability of three naturally occurring standardized extracts rich in polyphenols (*Vitis vinifera* [grape] seed extract, *Camellia sinensis* leaf [green tea] extract, *Quercus robur* [oak] wood/bark extract), both as single agents and as a multicomponent powder (Vitachelox[®]), to protect skin fibroblast culture against the toxic effect of polluting agents. Even when belonging to the same chemical class of polyphenols, the naturally occurring standardized extracts are characterized by different polyphenols, typically gallates in the green tea extract, leucocyanidins in the grape seed extract, and tannins in the oak wood extract.

Noteworthy, the multicomponent powder showed a synergistic effect, increasing cell viability over 77% (in comparison

with the positive control) in fibroblast cell cultures where environmental damage was experimentally induced. In addition, the multicomponent powder protected fibroblasts against the heavy metal-induced oxidative stress, by restoring the total amount of proteins and preserving the cellular components (lipids, proteins, and DNA).

In this *in vivo* study, the acute clinical application of the test product on healthy volunteers was evaluated by measuring prevention of any metal deposition within the stratum corneum (SC) following a 6-h exposure period in a polluted environment.

Patients and methods

Thirty healthy volunteers (mean age, 42±2 years; all women) living in the Milan greater area (the leading industrial and commercial city in Northern Italy, and one of the largest in Europe; n=15) or in the Beijing area (the capital of China; n=15) were recruited by a board of certified dermatologists enrolled by a third-party institution involved in the collection of data (Complife Group, Garbagnate Milanese, Milan, Italy). No differences between the two groups were disclosed in terms of baseline characteristics or concentration of metal ions (Table S1).

Written informed consent was obtained before baseline assessment, and the Ethical Committee of Complife group approved the study protocol.

According to the study protocol, only pH-7 detergents were used by volunteers to wash face skin during the 2 days prior to the study initiation. In a random manner, 2 mg/cm² of the multicomponent powder and of the placebo cream were applied on the hemi-faces of the enrolled participants, respectively. Then, participants were asked to spend six consecutive hours in a polluted area, according to their daily routine. SC samples of both hemi-faces were obtained before and after the exposure, using the tape stripping method (D-Squame[®], Cu Derm, Dallas, TX, USA).

The tape skin stripping method is a standardized procedure aimed at obtaining SC samples. In general, this method involves discarding the first strip and bringing the subsequent strips to –80°C prior to transferring five strips in a 50 mL test tube with 20 mL of HCl₃N. Then, the test tube is incubated slowly rotating or shaking at 37°C for 12–16 h. Following the incubation period, SC cells are separated by strips, and the solution is analyzed for the detection of heavy metals through atomic absorption spectroscopy.

Data were analyzed by descriptive statistics. The Student *t*-test was used for intragroup comparisons, with a *p* value <0.05 considered statistically significant.

Results

No statistically significant variations on the amount of heavy metals were found in the samples of SC cells obtained from the hemi-faces treated with the test product (Table 1). On the contrary, a significantly higher concentration of heavy metals was found in the SC cells samples of the hemi-faces treated with the placebo cream (Table 1). In detail, as illustrated in Figure 1, the following increases in heavy metals concentrations were detected: 95.5% increase in the concentration of chrome (Cr; $p < 0.001$), 130.2% increase in the concentration of iron (Fe; $p < 0.001$), 83.9% increase in the concentration of nickel (Ni; $p < 0.001$), and 142.6% increase in the concentration of zinc (Zn; $p < 0.001$). No differences between Milan and Beijing subgroups were reported (data not shown).

Table 1 Amount of heavy metals found in SC cells samples with the multicomponent powder or placebo

Ion	Multicomponent powder		Placebo	
	T=0 h	T=6 h	T=0 h	T=6 h
Cr (ppb)	43.5 (2.9)	46.4 (2.1)	44.9 (3.0)	87.8 (5.4)
Fe (ppm)	5.2 (0.3)	5.5 (0.5)	5.3 (0.3)	12.2 (0.7)
Ni (ppb)	6.3 (0.4)	5.9 (0.7)	6.2 (0.4)	11.4 (0.7)
Zn (ppb)	135.8 (8.3)	144.5 (7.9)	136.7 (7.6)	331.6 (16.3)

Note: Data are provided as mean (SE).

Abbreviations: Cr, chrome; Fe, iron; Ni, nickel; SC, stratum corneum; Zn, zinc.

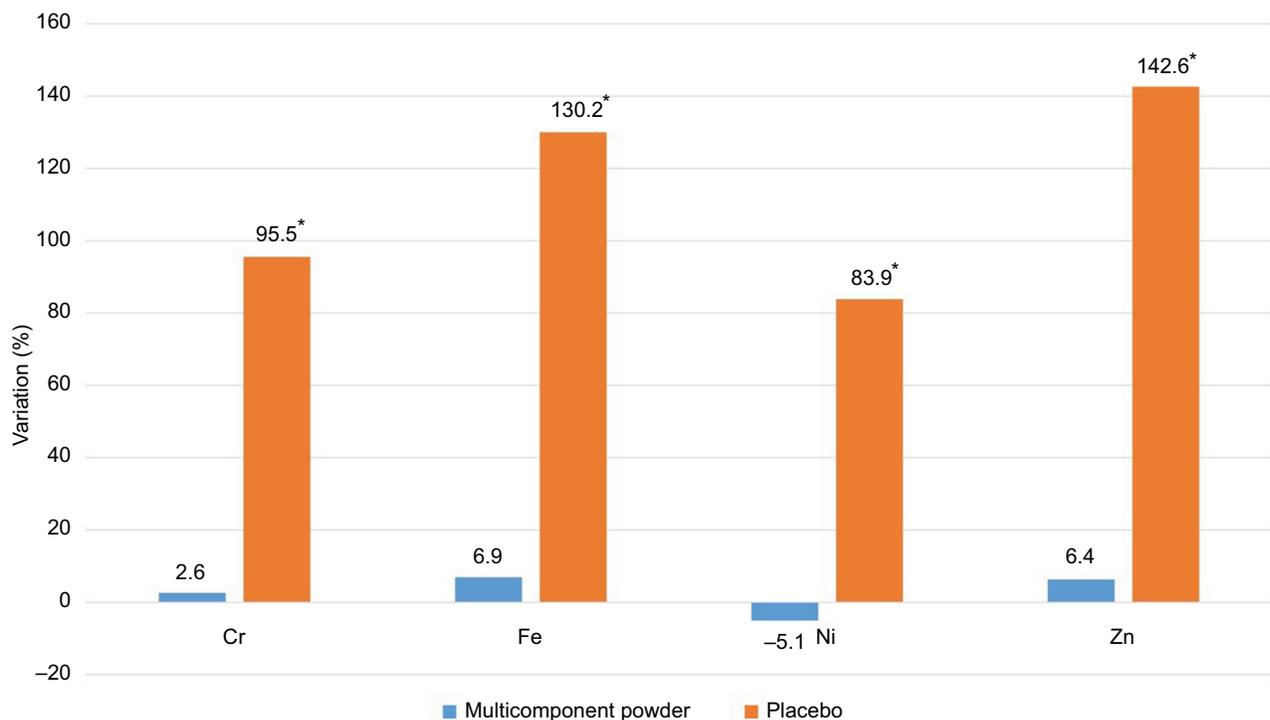


Figure 1 Variation (%) in the concentration of heavy metals in SC cells samples. * $p < 0.001$.

Abbreviations: Cr, chrome; Fe, iron; Ni, nickel; SC, stratum corneum; Zn, zinc.

Discussion

The skin is the single largest organ of the human body that provides protection from mechanical impacts and pressure, variations in temperature, microorganisms, radiation, and chemicals. However, the protective ability of the skin is not unlimited, and problems arise when an abnormal exposure to environmental stressors exceeds the skin's normal defensive potential.^{14,15} Among all endogenous and exogenous factors that can affect skin health, particularly PM promotes skin tissue damages and plays a role in the pathogenesis of skin diseases.⁷

In this study, the clinical application of a polyphenol-rich multicomponent powder resulted in a significantly lower metal deposition within the SC of the face skin of 30 healthy volunteers from two polluted areas (Milan and Beijing). Although with some limitations (e.g., low number of participants and lack of some other testing procedures such as patch test), the results from this in vivo study validate and extend to in vitro findings in humans indicating that the polyphenol-rich multicomponent powder protects fibroblast cell cultures against the toxic effect of heavy metals, preserving cell viability, protein synthesis, and the structure of cellular components such as lipids, proteins, and DNA.

The effectiveness of the multicomponent powder in protecting skin cells against air pollution relies on a broad

spectrum of well-recognized, pharmacological and therapeutic properties possessed by each single active ingredient, namely grape seed extract, green tea extract, and oak wood extract. In particular, grape seed extract contains several active compounds including flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidines, and the stilbene derivative resveratrol that exert antioxidative, antiinflammatory, and antimicrobial activities, thus protecting from the oxidant action of metals.¹⁶ Moreover, in experimental studies green tea extracts prevented iron accumulation.¹⁷ In addition, grape seed proanthocyanidins significantly inhibited UV-induced skin tumor development as well as suppression of immune system in in vitro and in vivo studies.¹⁸ Regarding the antioxidant properties of the green tea extract, these are mainly due to the catechin epigallocatechin-3-gallate – a biologically active polyphenolic compound of green tea that has both direct and indirect antioxidant properties at the cellular level, including scavenging of free radicals, inhibition of ROS-generating enzymes, and reduction of inflammatory cytokines, and above all, chelation of metal ions.^{19,20} Lastly, results from a recently published in vitro study²¹ showed that the methanol extracts of *Q. robur* (oak) bark extract have high radical scavenging capacity, elastase, collagenase, tyrosinase inhibitory activities, and antibacterial activity against *Staphylococcus aureus*.

Conclusion

The findings of our study indicate that the multicomponent powder allows the prevention of any metal deposition within the SC following exposure in a polluted environment, suggesting that it could play an effective role in counteracting skin damages induced by air pollution.

Disclosure

ST, MM, and GM are employees of Indena S.p.A. LG is a consultant of Indena S.p.A. The authors report no other conflicts of interest in this work.

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Supplementary material

Table S1 Baseline characteristics and concentration of metal ions in the Milan subgroup (n=15) and in the Beijing subgroup (n=15)

Parameter	Beijing (n=15)	Milan (n=15)	Overall (N=30)
Age (years)	42.0±3.4	42.7±3.7	42.3±2.5
Gender (female)	15 (100%)	15 (100%)	30 (100%)
Basal heavy metals skin content			
Chromium	50.4 (3.6)	36.7 (4.0)	43.5 (2.9)
Iron	6.0 (0.4)	4.3 (0.5)	5.2 (0.3)
Nickel	7.1 (0.7)	5.5 (0.3)	6.3 (0.4)
Zinc	151.6 (12.0)	119.9 (10.4)	135.8 (8.3)

Notes: No differences were reported between the two subgroups in any parameter. Data are means (SE) or numbers (%).

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