Effect of a nutritional supplement in the protection against keratinocyte damage due to pollution: *in vitro* confirmation

Efeito de um suplemento nutricional na proteção contra os danos decorrentes da poluição em queratinócitos: comprovação in vitro

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ABSTRACT

Introduction: The interaction between the skin and air pollutants has demonstrated effects in the cutaneous barrier, as well as triggering oxidative processes related to premature ageing of the skin. The aryl hydrocarbon receptor (ArH) is a transcription protein that interacts with xenobiotics, regulating the transcription of genes involved with oxidative stress, inflammation, immunosuppression and pigmentation, besides leading to processes related to ageing and carcinogenesis.

Objective: Evaluate the anti-pollution efficacy of an antioxidant association for the prevention of the nuclear translocation of the AhR receptor.

Methods: A in vitro model (keratinocyte culture) was exposed to cigarette smoke and the presence of AhR was measured through sanduwich ELISA assay.

Results: The treated culture demonstrated inhibition of the nuclear translocation of AhR in all concentrations evaluated: ArH increase of 75.38%; 59.88% and 117.79% are seen with the concentrations of 0.316; 0.100 and 0.0316mg / mL, respectively.

Conclusion: The results suggest the ability of the formulation analyzed in preventing the activation of genes responsible for the damaging effects of cigarette smoke.

Keywords: DNA Damage; Air Pollution; Aryl Hydrocarbon Receptor Nuclear Translocator

RESUMO

Introdução: *A interação da pele com poluentes atmosféricos tem demonstrado efeitos na barreira cutânea, assim como o desencadeamento de processos oxidativos relacionados ao envelhecimento prematuro da pele. O receptor de aril hidrocarbonetos (ArH) é proteína de transcrição que interage com os xenobióticos, regulando a transcrição de genes envolvidos com estresse oxidativo, inflamação, imunossupressão e pigmentação, além de levar a processos relacionados ao envelhecimento e carcinogênese.*

Objetivo: *Avaliar a eficácia antipoluente de uma associação antioxidante na prevenção da translocação nuclear do receptor AhR*

Métodos: *Um modelo in vitro (cultura de queratinócitos) foi exposto à fumaça de cigarro, e a presença de AhR foi medida por ensaio Elisa-sanduíche.*

Resultados: *A cultura tratada demonstrou inibição da translocação nuclear do AhR em todas as concentrações avaliadas: aumentos de ArH de 75,38%; 59,88% e 117,79% são observados nas concentrações de 0,316; 0,100 e 0,0316mg/ml, respectivamente.*

Conclusão: *Os resultados sugerem a capacidade da formulação avaliada em prevenir a ativação de genes responsáveis pelos efeitos nocivos da fumaça de cigarro.*

Palavras-chave: *Dano ao DNA; Poluição do Ar; Translocador Nuclear Receptor Aril Hidrocarboneto*

Original Articles

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INTRODUCTION

There is consistent evidence that air pollution has deleterious effects on human health, with respiratory and cardiovascular repercussions. More recently, the interaction of these pollutants with the skin has also shown effects on the cutaneous barrier,[1] in addition to triggering oxidative processes related to premature skin aging, as demonstrated in a cohort study in large urban centers in Germany.[2]

These mechanisms of damage involve the expression of proinflammatory mediators and AhR (aryl hydrocarbon receptor) translocation protein, acting on both keratinocytes and melanocytes, leading to increased production of oxygen-free species.[3,4]

The AhR receptor is a transcription protein that interacts with xenobiotics, which are chemical compounds foreign to the body. This interaction with xenobiotics is similar to that of hormone receptors. The binding of a xenobiotic compound activates the receptor, allowing its translocation to the nucleus, which regulates the transcription of genes involved with oxidative stress, inflammation, immunosuppression, and pigmentation, therefore being capable to lead to premature aging and carcinogenesis processes. Inhibition of AhR-mediated gene activation could significantly interfere with signs of aging and carcinogenesis.[4,5]

Cigarette smoke contains over 4,000 toxic compounds, including many polycyclic aromatic hydrocarbons (PAHs), dioxins and furans that exert harmful effects on the skin by the induction of genes that act on inflammation and oxidation,[6,7] being able to activate the translocation of AhR.[8,9]

Exposure to the xenobiotic agents contained in the pollutants (e.g. cigarette smoke) can reduce (*in vitro*) the presence of cytoplasmic AhR, since this receptor, once activated by the agent (e.g. PAH), would migrate to the nucleus aiming at activating the genes linked to inflammation and oxidation.[10]

Vanzo *et al.*[11] investigated the translocation inhibition effect in keratinocyte culture in 2015. A strategy aimed at preventing damage caused by constant exposure to exogenous agents – in particular cigarette smoke – involves the use of substances that inhibit the translocation of AhR to the nucleus, thus avoiding imbalance of cutaneous cellular homeostasis and the progression of reactions that oxidize organic substrates.

OBJECTIVE

To evaluate the anti-pollutant efficacy of a nutrient association in the prevention of nuclear translocation of the aryl hydrocarbon receptor (AhR), induced in an *in vitro* model (culture of keratinocytes) by exposure to cigarette smoke.

MATERIALS AND METHOD

This trial was carried out in HaCat human keratinocyte culture, purchased from the State of Rio de Janeiro's Cell Bank, Rio de Janeiro (RJ), Brazil.

The cultures were incubated at three previously determined non-cytotoxic concentrations of the Exímia Temporize® formulation (Melora-FQM, Rio de Janeiro, Brazil), consisting of the vitamins E and C, beta-carotene, lutein, lycopene, linseed oil, zinc and selenium. The concentrations were 0.316, 0.100 and 0.0316 mg/ml, respectively, and the cells were kept in touch with the formulation for 48 hours.

After 48 hours of treatment, the cultures were exposed to cigarette smoke using an appropriate chamber that allowed the complete combustion of two cigarettes. The cells were incubated for an additional 24 hours with the evaluated formulation. After this period, they underwent extraction of cytoplasmic lysate (three cycles of freezing at -20 °C, with a 20 minute interval between each cycle, and subsequent centrifugation at 10,000 rpm for 10 minutes), followed by the quantification of the mediator AhR.

AhR measurement

The presence of AhR was measured using the sandwich-Elisa (Enzyme Linked ImmunoSorbent Assay) using commercially available kits (Uscn Life Science Inc., Houston, TX, USA). The absorbance reading was performed using a Multiskan® GO® monochromator. AhR values were normalized based on the total protein of the sample measured using the Bradford technique.[12]

To analyze the results, the statistical evaluation used the ANOVA test, which allowed measuring the data's variation base on comparisons between the groups, followed by the Bonferroni post-test. A 5% significance level (GraphPad Prism v6) was used.

RESULTS

Exposure to cigarette smoke *versus* not-exposed control

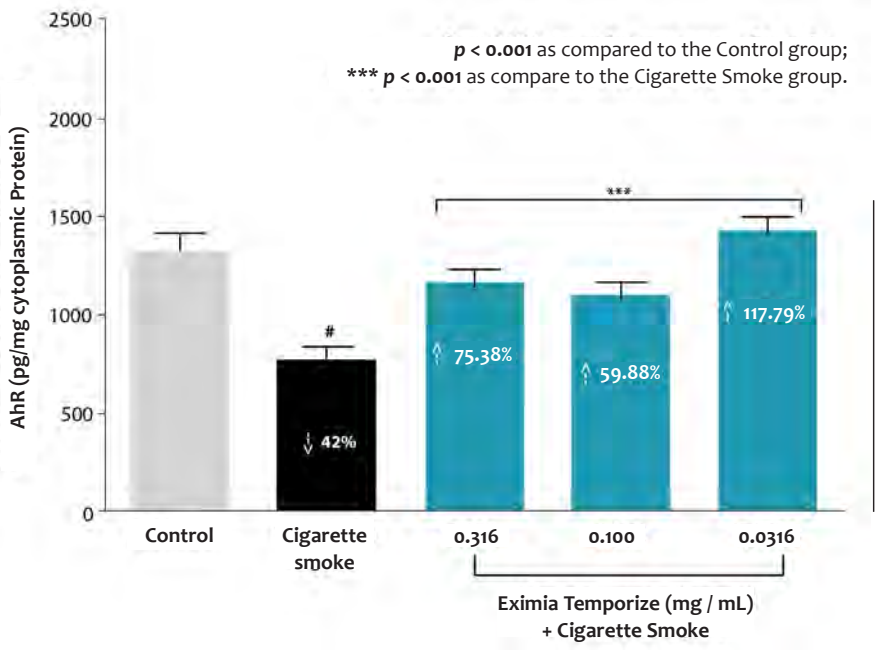
Exposure of cells to cigarette smoke promoted a 42% reduction in AhR availability in the cytoplasm of the cell when compared to baseline control ($P < 0.001$). This result indicates a possible migration of the AhR to the cell nucleus that is coupled to the xenobiotic agent.

Cultures previously treated with the assessed formulation *versus* untreated control exposed to cigarette smoke

Pretreatment of the cultures with the evaluated formulation was shown to inhibit AhR nuclear translocation at all concentrations evaluated, with the inhibition value reaching a level higher than 100% as compared to the cells exposed to cigarette smoke. Increases of 7.38%, 59.88% and 117.79% were observed at the concentrations of 0.316; 0.100 and 0.0316 mg/ml, respectively. The results suggest the evaluated formulation has the ability to prevent the activation of the genes responsible for the damaging effects of cigarette smoke (Graph 1).

DISCUSSION

Cigarette smoke exerts harmful effects on the skin that are mediated by the AhR receptor, which is a cytosolic transcription factor found in its inactive form, which binds to the toxic agent and translocates it to the cell nucleus. In the nucleus, AhR regulates the transcription of genes involved in oxidative



Graph 1: AhR quantification in culture of human keratinocytes treated with the test formulation as compared to the controls: without exposure and treatment, with normal levels of AhR (gray column), and exposed to smoke however untreated (black column), with AhR reduction, due to nuclear translocation. Note the protective effect of the test formulation, close to normal levels of receptors, even when exposed to the pollutant stimulus

stress, inflammation, immunosuppression, pigmentation, premature aging and carcinogenesis.[13]

In addition to mediating these mechanisms at the nuclear level, AhR can also be regulated by oxidative mechanisms. Therefore, inhibiting AhR translocation would prevent damage caused by the xenobiotics in the cell nucleus.[14]

In order to analyze AhR's involvement in cutaneous aging caused by cigarette smoke, in 2008 Ono *et al.*[10] exposed primary human fibroblasts to a tobacco smoke extract, observing increased induction of mRNA for metalloproteinase 1, associated with greater expression of cytochrome P1B1 (CYP P1B1).

The present study has demonstrated that the treatment of keratinocytes with the association of nutrients avoided nuclear translocation, keeping the AhR receptors in the cytoplasm. The prevention of this translocation – characterized by the increased concentration of these receptors – reached 117.79% as compared to the area that had not received any treatment before having been exposed to cigarette smoke.

CONCLUSION

The association of nutrients contained in the evaluated formulation exerted cellular protective effect against the damage caused by xenobiotic pollutants to keratinocytes, since it protected the nuclear translocation of the AhR receptor, reaching a value in excess of 100% regarding the control. These findings demonstrate that the evaluated treatment has adjuvant action in the prevention and treatment of the skin aging process due to extrinsic factors, which is represented in the present study by cigarette smoke. ●

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DECLARATION OF PARTICIPATION:

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Conduction of the study, data compilation and review, oversight of the statistical evaluation, preparation of the manuscript.