Study of the anti-pollution potential of olive leaf extract *(Olea europaea)* by Aryl hydrocarbon receptor (AhR) modulation in the skin.

Accioli, C.A.F.[1*]; Mendes, G.E.M.[2]; Bello, M.L.[2]; Santos, B.A.M.C; Rodrigues, C.R.[1].

[1] *Universidade Federal do Rio de Janeiro/Programa de Pós-Graduação em Ciências Farmacêuticas, Cidade Universitária, Rio de Janeiro, RJ, Brazil.*
[2] *Universidade Federal Fluminense/Programa de Pós-Graduação em Ciências e Biotecnologia, Niterói, RJ, Brazil.*
caf.ufrj@gmail.com

Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor, expressed in different tissues, involved in environmental aggressors responses, such as atmospheric pollution. From the interaction with an agonist, AhR releases its cytosolic complex, translocates to the nucleus, altering the expression of target genes. Its activation also triggers non-genomic signals and together, these responses culminate in oxidative stress and inflammation [1]. Studies already associate the development of respiratory, cardiovascular, neurodegenerative diseases and cancers with exposure to environmental factors, mediated by AhR. Due to its extension and direct contact with the environment, the skin is affected by these compounds, from pollution, cutaneous microbiota and solar radiation photoproducts, leading to skin aging [2], inflammatory diseases [3] and even skin cancer [2]. In contrast, recent studies have shown that synthetic [4] and natural compounds (polyphenols) [5] are able to modulate AhR activation, minimizing the harmful response of these aggressors in the skin. Thus, AhR constitutes a new and interesting therapeutic target for the prevention or treatment of skin conditions associated with the exposome. Despite this, AhR is a still poorly explored target. It is known that olive leaf extract (*O. europaea*) is rich in polyphenols and has shown photoprotective and antioxidant efficacy in previous studies [6]. Thus, the aim of the study was to investigate its potential as a modulator of AhR activation for cutaneous use, aiming to prevent pollution induced skin damage.

Material and Methods

In vitro antipollution efficacy: Human keratinocytes were incubated with 3 non cytotoxic concentrations of olive leaf extract for 72h in the presence of Benzo[a]pyrene - B[a]P (1µM). After 24 hours, the cell lysate was collected for quantification of cytoplasmic AhR by ELISA assay. Phytochemical analysis: The phenolic composition of the extract was characterized by UHPLC-MS/MS. *In silico* studies: The structure of the human HSP90-XAP2-AhR complex (PDB: 7ZUB) was used as a template for the model constructed in the study. Molecular dynamic (MD) simulations during 300ns were performed with the model in its apo form using Amber package, FF19SB force field, and Protein clustering was also performed using K-means algorithm. The binding mode of the identified compounds and AhR was evaluated by molecular docking using moldock score at Molegro virtual docker software. AhR-ligand complexes of Oleuropein and pollutant (B[a]P) were also analyzed by MD simulations, in a 100ns trajectory and ΔG binding were estimated by MM-PBSA calculations based on the last 5ns of trajectory.

Results and Discussion

In the in vitro study, B[a]P exposed group exhibit a reduction of 53% in cytoplasmic AhR levels, indicating AhR activation and nuclear translocation induced by pollutant. In cultures treated with olive extract and exposed to B[a]P, the protective effect of the extract was observed at concentrations of 0.32 and 0.10 µg/mL, which significantly prevented AhR activation by 40% and 33%, respectively, compared to the control exposed only to B[a]P. Oleuropein has been identified as the major compound in UHPLC-MS/MS analysis. The other identified phenolics, in decreasing order of relative content, were: Oleuropein (isomers 1 and 2); Verbascoside, Luteolin glucoside (isomer 1), Ligustroside, Oleoside, Luteolin glucoside (isomer 2), Apigenin-7-*O*-glucoside, Quercetin, Hydroxytyrosol glucoside and Kaempferol. In silico studies showed a good affinity of the identified compounds with the AhR binding

site, PAS-B, showing a similar binding mode of known ligands, in the cavity in molecular docking study. For Oleuropein, interactions were shown to be stable over time in the molecular dynamics simulation, exhibiting a favorable ΔG binding, estimated by MM-PBSA, compared with B[a]P, reinforcing the molecular docking results. These data suggest a probable competition of oleuropein with pollutants for binding to AhR, thus modulating receptor activation and its consequent cellular responses.

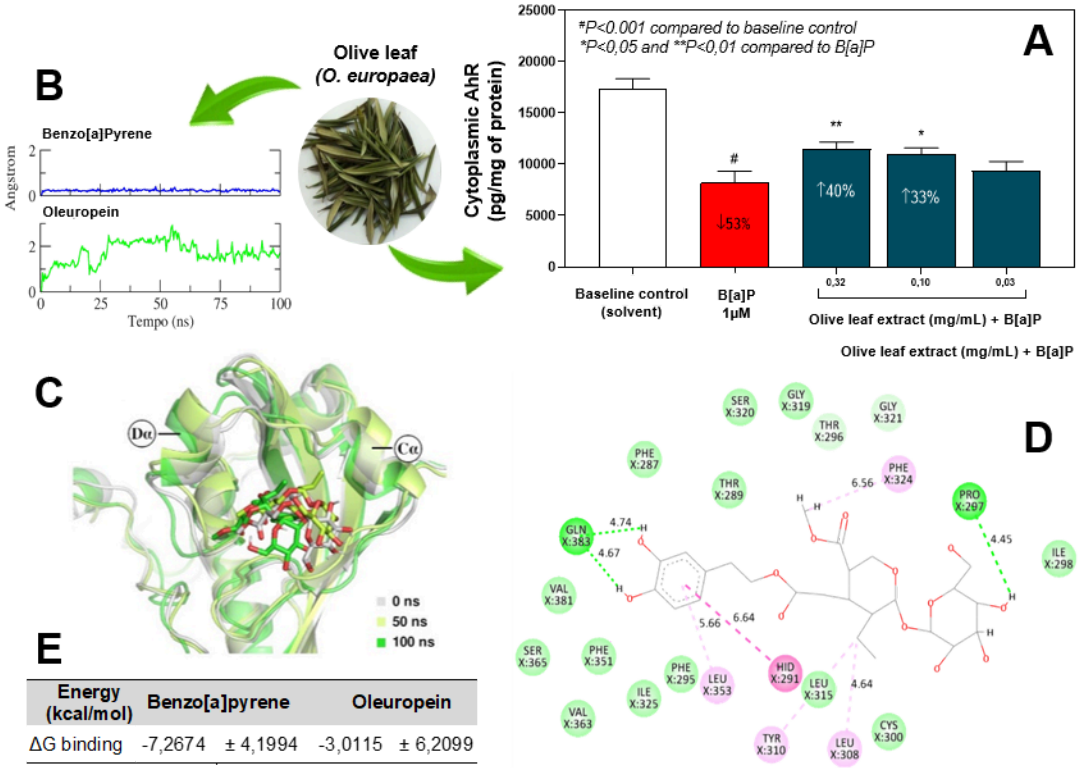

Figure 1. A. Quantification of cytoplasmic AhR in keratinocytes exposed to B[a]P and treated with olive leaf extract**. B.** RMSD of bound B[a]P and oleuropein. **C.**Binding mode of oleuropein-AhR complex at 0, 50 and 100 ns of the MD simulation trajectory. **D.**2D representation of intermolecular interactions identified at the end of MD. **E.** ΔG binding of complexes estimated for the last 5ns of trajectory by MM-PBSA.

Conclusion

Olive leaf extract (*O. europaea)* showed potential for dermatological use against damage induced by environmental factors, especially pollution, via modulation of the Ah receptor. Additionally to its known photoprotective and antioxidant activities, this extract stands out as a complete active ingredient for the skin. Despite this, the data are still preliminary, and further studies with the extract and cosmetic formulation containing the extract are needed to confirm its anti-pollution activity.

Acknowledgments

The authors thank Simony Mendonça and the *analytical center of DPNA-FF/UFRJ* for the phytochemical analysis of the olive leaf extract by UHPLC-MS/MS. They also thank Michelle Silva and *Medcinvitro* for performing the cellular assays.

Bibliographic References

[1] C. Esser en A. Rannug, "The Aryl Hydrocarbon Receptor in Barrier Organ Physiology, Immunology, and Toxicology", *Pharmacol. Rev.*, vol 67, no 2, 2015, doi: 10.1161/CIRCULATIONAHA.115.017465.

[2] C. Vogeley, C. Esser, T. Tüting, J. Krutmann, en T. Haarmann-Stemmann, "Role of the aryl hydrocarbon receptor in environmentally induced skin aging and skin carcinogenesis", *Int. J. Mol. Sci.*, vol 20, no 23, 2019, doi: 10.3390/ijms20236005.

[3] S. E. Mancebo en S. Q. Wang, "Recognizing the impact of ambient air pollution on skin health", *J. Eur. Acad. Dermatology Venereol.*, vol 29, no 12, 2015, doi: 10.1111/jdv.13250.

[4] J. Tigges *et al.*, "The New Aryl Hydrocarbon Receptor Antagonist E/Z-2-Benzylindene-5,6-Dimethoxy-3,3-Dimethylindan-1-One Protects against UVB-Induced Signal Transduction", *J Invest Dermatol*, vol 134, no 2, 2014, doi: 10.1038/jid.2013.362.The.

[5] Z. Xue *et al.*, "Mechanisms and therapeutic prospects of polyphenols as modulators of the aryl hydrocarbon receptor", *Food Funct.*, vol 8, no 4, 2017, doi: 10.1039/c6fo01810f.

[6] A. C. P. da Silva *et al.*, "Photoprotection assessment of olive (Olea europaea L.) leaves extract standardized to oleuropein: In vitro and in silico approach for improved sunscreens", *J. Photochem. Photobiol. B Biol.*, vol 193, 2019, doi: 10.1016/j.jphotobiol.2019.03.003.