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Effect of Endocrine Disrupting Chemicals on gut microbiota in the *in vitro* model of Enteric Nervous System

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Introduction

In recent years, the intestinal microbiota has become a key element in understanding the bidirectional communication of the gut-brain axis (GBA) (1-3). In this axis, the role of the enteric nervous system (ENS) is crucial since it serves as a link between the intestine and the brain. In our daily life, humans are exposed to wide variety of environmental pollutants, such as endocrine-disrupting chemicals (EDCs). These compounds enter our body mainly through the daily intake of food, and reach the gut microbiota, altering it and causing neurotoxicity via the GBA. Therefore, the microbiota is decisive for the toxicological risk assessment (4).

The main objective of this project is to study how the dysbiosis of the intestinal microbiota caused by exposure to endocrine disruptors is related to neuronal disorders using an *in vitro* model.

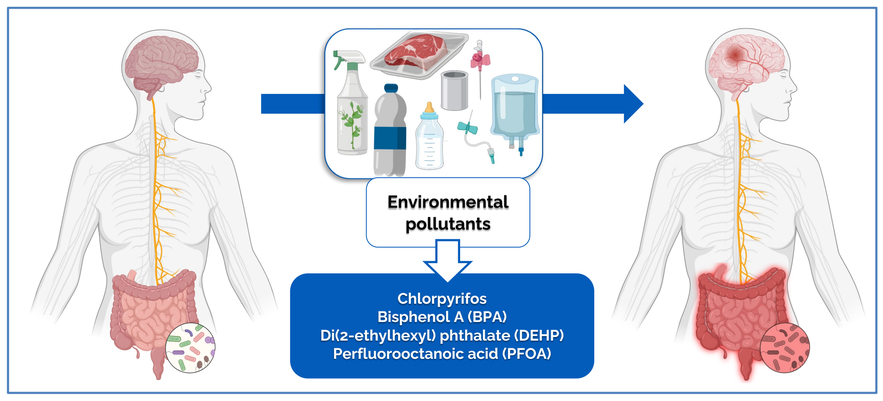
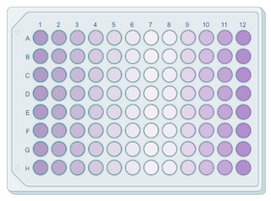


Fig.1 *- Scheme of the relationship between EDCs exposure and gut microbiota dysbiosis*

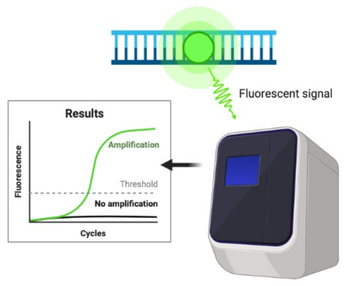
Materials and methods

- Cell culture: Caco-2 and HT-29 cells are growing for 21 days in DMEM. SH-SY5Y cells grow 7 days on Ham's F-12K.
- SH-SY5Y Differentiation: Retinoic Acid is added to the culture medium.
- Bacterial culture: *Enterococcus faecalis, Escherichia coli, Streptococcus salivarius, Streptococcus mitis, Lactobacillus plantarum, Lacticaseibacillus rhamnosus* grow on Mueller-Hinton Agar and BHI Agar plates.

MTT assay (Viability test)



Genetic expression of *BDNF, TUBB3, Casp3* and *Wnt3a*
- Real-time PCR of some genes involved in growth, survival, maturation and correct neuronal function.



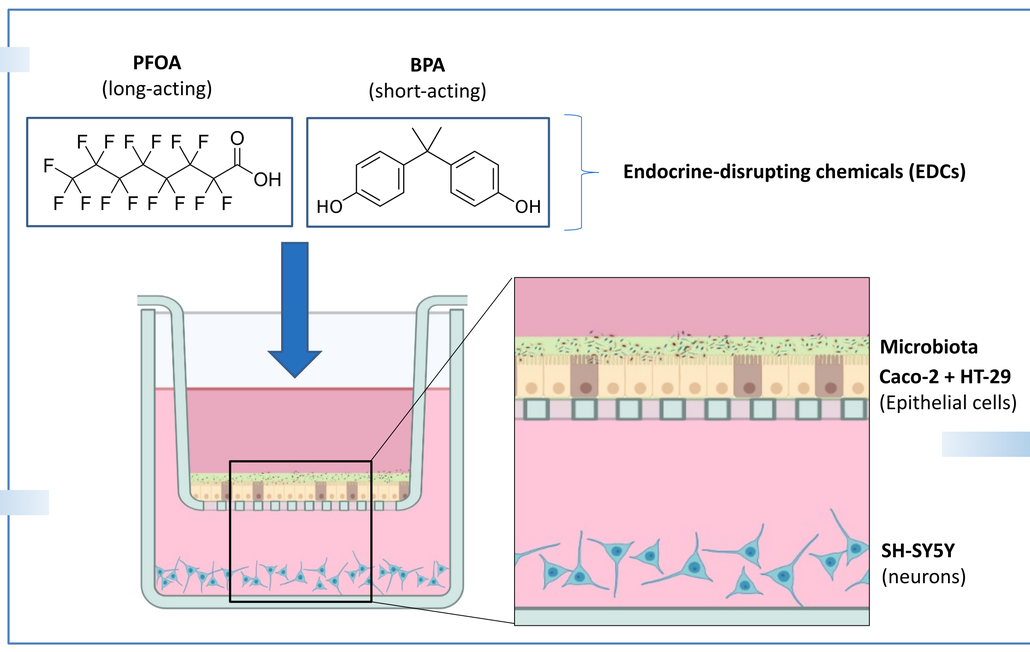
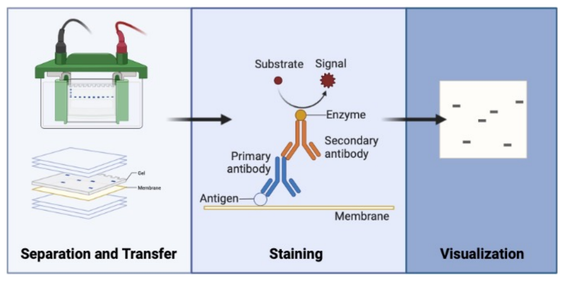


Fig.2 *- Perfluorooctanoic acid and Bisphenol A treatment on in vitro gut-ENS model (Transwell) and methods workflow.*

Nrf2 Activation (Protein expression)
- Western Blot technique.



Preliminary results

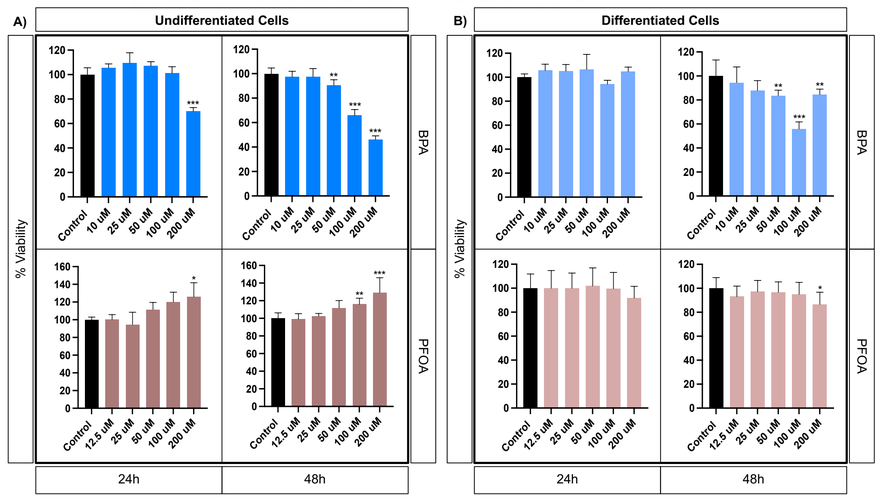


Fig.3 *- Cell viability by MTT test. A) Exposure at 24h and 48h to BPA and PFOA in undifferentiated SH-SY5Y cells. B) Exposure at 24h and 48h to BPA and PFOA in differentiated cells. One-way ANOVA Statistical analysis performed using Bonferroni's correction (*p< 0.05, **p< 0.01, ***<0.001).*

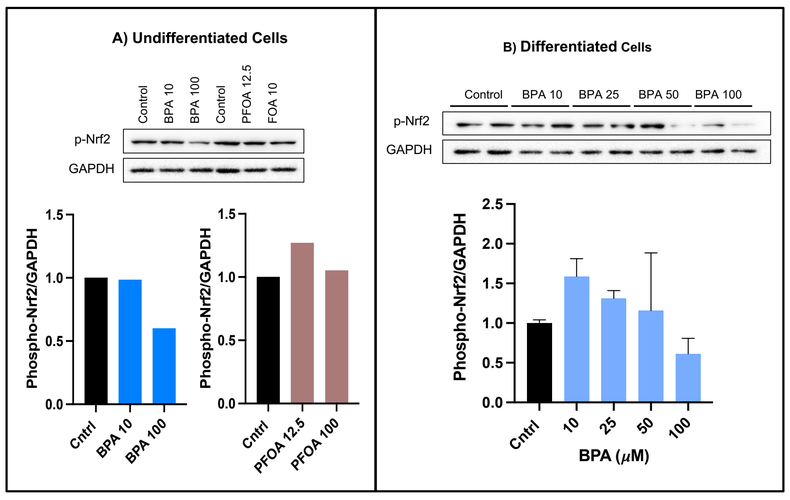


Fig.4 *- Nrf2 levels by Western Blot. A) Nrf2 activation after treatment with BPA (10 and 100μM) and PFOA (12.5 and 100 μM) in undifferentiated SH-SY5Y cells. B) Nrf2 activation after treatment with BPA (10 and 100μM) in undifferentiated SH-SY5Y cells. Data are expressed as mean and S.E.M.*

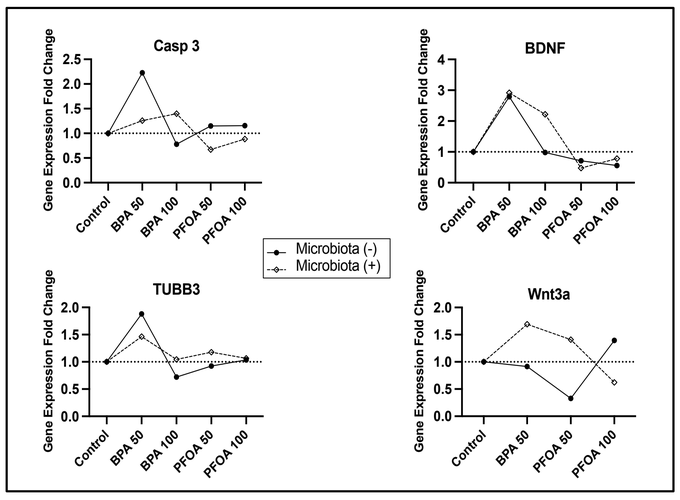


Fig.5 *- A) Relative expression of Wnt3a, TUBB3, Casp3 (Caspase 3) and BDNF after exposure to BPA (50 and 100μM) and PFOA (50 and 100 μM) in differentiated SH-SY5Y cells with and without microbiota. In the results shown, the species used have been: L.plantarum and L. rhamnosus at 10^3 CFU/mL.*

- Differentiated SH-SY5Y cells are more resistant to BPA exposure (Fig.3). The increase in viability observed in undifferentiated cells exposed to PFOA, may be due to an increase in mitochondrial metabolism (Fig.3A).
- The decrease in Nrf2 levels in cells exposed to BPA indicates a down-regulation of the Nrf2 transcription response (Fig.4)(5).
- The results of the PCR assay, show that microbiota may have a positive effect on the cells. *Caspase 3*, which is one of the enzymes involved in the apoptosis process, is reduced in cells exposed to PFOA co-cultured with bacteria. *TUBB3*, a gene associated with neuronal projection and dendrite growth, present higher values in samples with bacteria after exposure to BPA and PFOA (Fig.5).

Conclusions

The described *in vitro* model help in providing the mechanistic insights about crosstalk between EDCs-microbiota-nervous system which are associated with a variety of neurodegenerative disorders. The results show that cell differentiation is an improvement and the possible beneficial effect of *L.plantarum and L. rhamnosus* on SH-SY5Y cells after BPA/PFOA exposure. Further, such platform can provide an affordable and time-saving methodology reducing the use of animals for toxicity testing of environmental chemicals.

References

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