In vitro modulation of estrogen receptor activity by selected drugs and polystyrene micro- and nanoparticles

Lucija Božičević[1], Krunoslav Ilić[1], Ivan Pavičić[1], Ivana Vinković Vrček[1]

(1) Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10000 Zagreb, Croatia

Introduction

Endocrine disruptors (EDCs) are defined as an exogenous chemicals or mixture of chemicals that can interfere with any aspect of the hormones action. The Endocrine Society has claimed that EDCs affect male and female reproduction, neurodevelopment, thyroid function, development of various cancers like breast and prostate cancer. This study investigated effect of several commonly used pharmaceuticals that are suspected to have EDC properties (**paracetamol, ibuprofen, fluoxetine and carbamazepine**) and polystyrene micro- and nanoparticles (**PSMPs and PSNPs**) on estrogen receptor activity using T47D-KBluc cell line after **48 and 72 hours treatments**.

Methods

- **MTS cell viability assay** using CellTiter 96® AQueous One Solution Cell Proliferation Assay kit and DMSO treatment as a positive control.
- **Cell viability and apoptosis induction** by Annexin V-FITC Assay Kit using DMSO treatment as a control for dead cells and staurosporine treatment as a control for apoptosis induction.
- **Estrogen receptor activity** was determined by **Luciferase Assay** Kit according to modified procedure described for the VM7Luc4E2 assay of OECD Test Guideline TG 455.

Figure 1. Cell viability presented as % of live cells compared to negative control (untreated cells - Ctrl) after 48 hours (light green, left columns) and 72 hours (dark green, right columns) treatment with (a) paracetamol, (b) ibuprofen, (c) carbamazepine and (d) fluoxetine. Concentrations of drugs are in μM. Positive controls were cells treated with 10% DMSO.

Figure 2. Percentage of dead, live, early apoptotic (EA) and late apoptotic (LA) cells treated with (P) paracetamol, (I) ibuprofen, (C) carbamazepine and (F) fluoxetine after 48 hours (a) and 72 hours (b) as compared to control (untreated - Ctrl) cells. Concentrations of drugs are in μM. Positive controls for dead cells were cells treated with 10% DMSO, while positive control for apoptosis induction were cells treated with staurosporine.

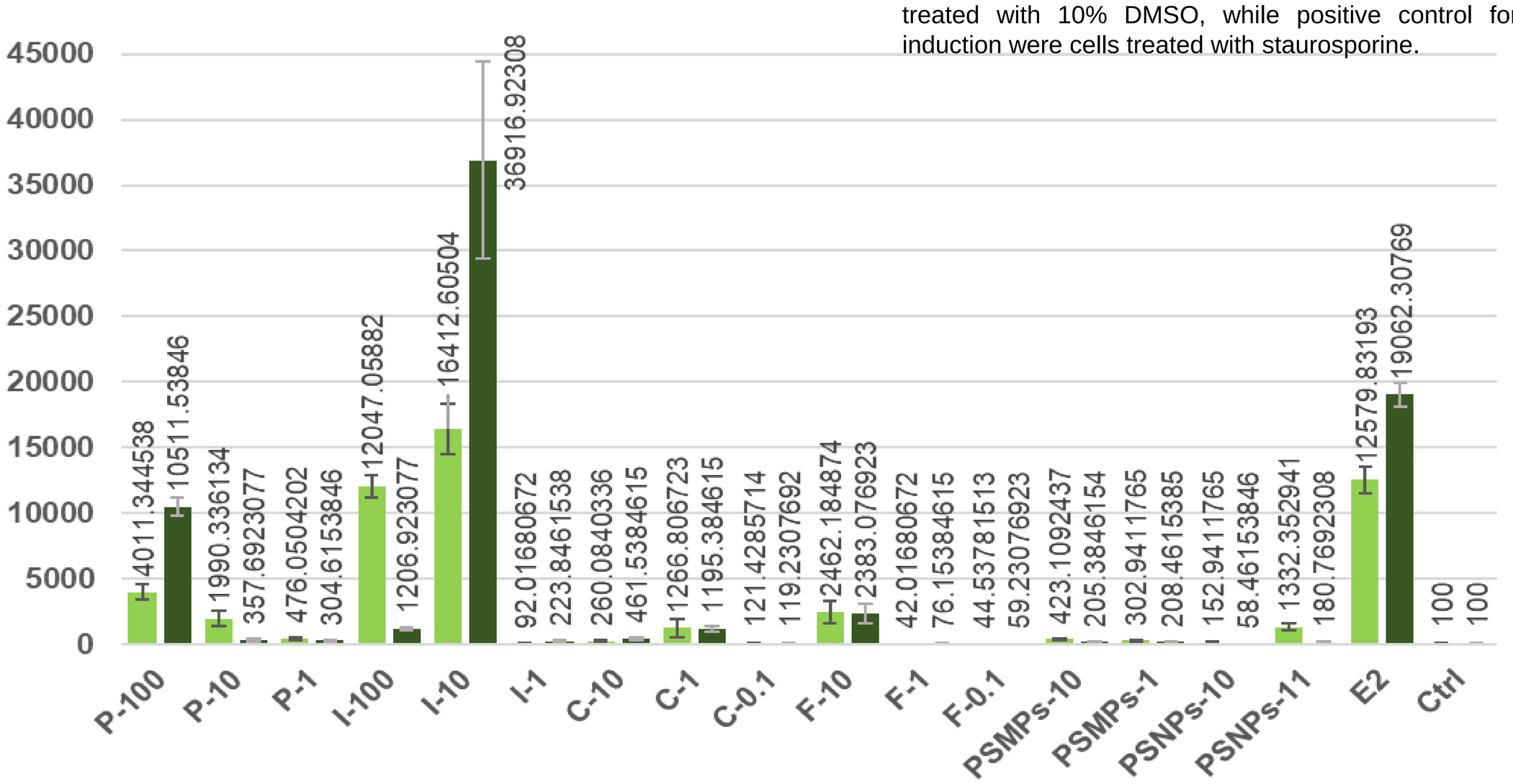

Figure 3. The effects of paracetamol (P), ibuprofen (I), carbamazepine (C), fluoxetine (F) and polystyrene micro- (PSMPs) and nanoparticles (PSNPs) on estrogen receptor activation after 48 hours (light green, left columns) and 72 hours (dark green, right columns). The results are presented as % of control (untreated – Ctrl) cells, while treatment with 17-β estradiol (E2) was used as a positive control. Concentrations of drugs are in μM. Concentrations of PSMPs and PSNPs are in ppm.

Conclusions

- **Concentrations chosen for Luciferase Assay measurements were proven to be non-toxic for cells. Concentration of PSMPs and PSNPs that were used in the experiment were proven non-toxic in previous experiments from our group.**
- **Paracetamol and ibuprofen show estrogenic effects, while fluoxetine shows anti-estrogenic effects.**
- **Further experiments are needed to estimate if the dose-response curves for these drugs are U-shaped, which is characteristic for endocrine disruptors. Mixtures of drugs with NPs/MPs will be tested as well.**

lbozicevic@imi.hr

References:

- Roje, Ž., Ilić, K., Galić, E., Pavičić, I., Turčić, P., Stanec, Z., Vinković Vrček, I. (2019) Synergistic effects of parabens and plastic nanoparticles on proliferation of human breast cancer cells. Archive of Industrial Hygiene and Toxicology 2019;70(4):310-314
- Vandenberg LN. Non-monotonic dose responses in studies of endocrine disrupting chemicals: bisphenol a as a case study. Dose Response. 2013;12(2):259-276.