ERβ and NFKB partcipate in the mechanism of action of AOH in SKOV3 cells

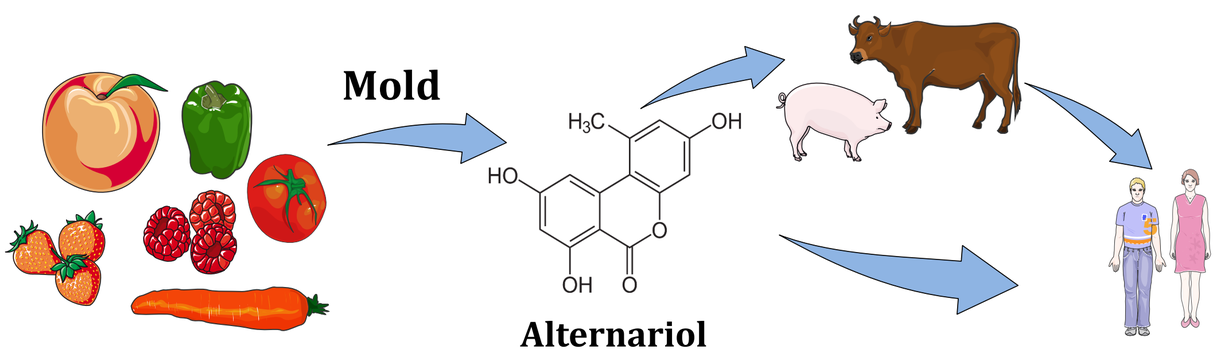
Marta Justyna Kozieł[1], Karolina Kowalska[1], Agnieszka Wanda Piastowska-Ciesielska[1#]

[1]Department of Cell Cultures and Genomic Analysis, Medical University of Lodz
[#] corresponding author: agnieszka.piastowska@umed.lodz.pl

Introduction:

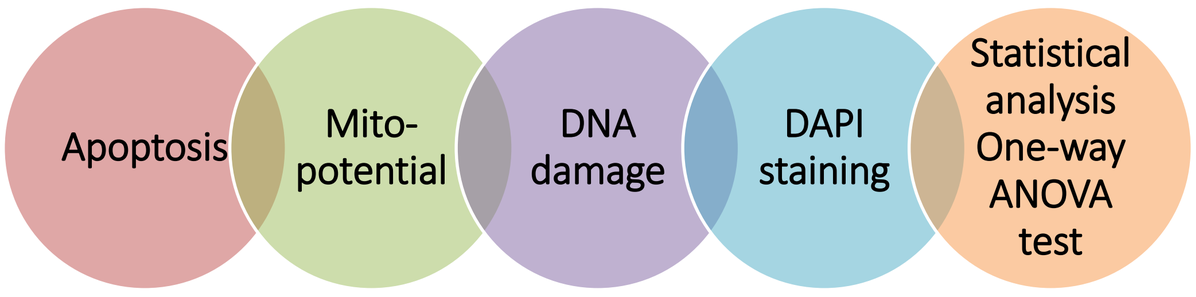
Mycotoxins are toxic secondary metabolites of fungi produced mainly by *Fusarium, Alternaria, Asperegillus* and *Penicillium* species. It is generally known that mycotoxins might trigger a negative effect both on human and animal health but their detailed impact on human health is still under investigation. Due to structural similarity to naturally occurring estrogen, mycotoxins might affect human endocrine system via binding to the estrogen receptors (ERs). It may result in, for instance, increased growth or proliferation of cells. Zearalenone (ZEA) and alternariol (AOH) are considered as estrogenic mycotoxins, whereas ZEA estrogenicity is proved, AOH is still not investigated.

The aim of the study was to assess the role of the estrogen receptor ERβ and the transcription factor NFKB in response to the action of an estrogenic mycotoxin (AOH).



Material and methods:

Cells were treated with AOH (10μM), ERβ (PHTPP) and NFKB inhibitors (BAY 11-7082). Apoptosis was evaluated with Muse Annexin V & Dead Cell Kit, mitopotential with Muse MitoPotential Kit , DNA damage with Muse Multi-Color DNA Damage Kit. Western blot was used to detect proteins. DAPI staining was done to visualize nuclei of cells.



Results:

AOH induced apoptosis (*Figure 1*), depolarization of the mitochondria (*Figure 2*) and DNA damage in SKOV3 cells (*Figure 3*). Moreover these effects were associated with the blocking of ERβ and NFKB

Scientific achivements:

- Participation in international conferences
- Co-author of published article
- Obtaining the Rector's research grant
- Obtaining funding under the "BRaIn Internal Grants Program"

Results:

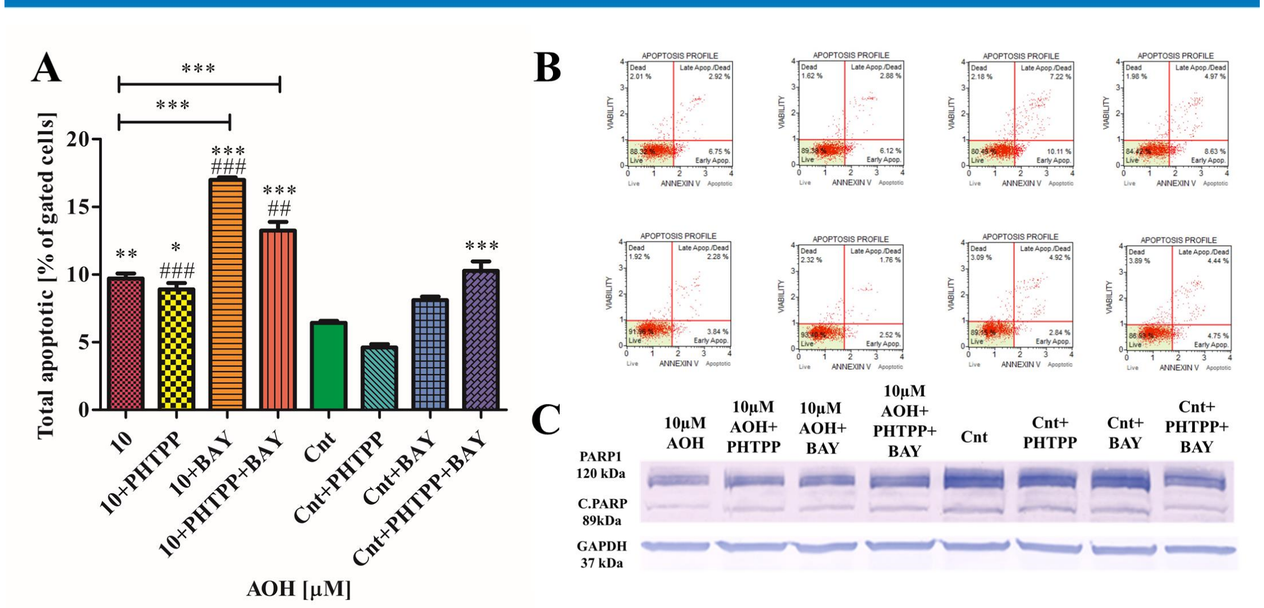


Figure 1. Alternariol induces apoptosis ovarian cancer cells. A- Apoptosis in the SKOV3 cell line woth the representative results B. C- The representative results of western blot for PARP1, C.PARP and GAPDH in SKOV3 cells. Results are expressed as mean±SE, **p<0.05*, ***p<0.01*, ****p<0.001* as compared to control. * refers to non-treated cells, # to adequate positive control. AOH- alternariol, Cnt- control, PHTPP- selective ERβ inhibitor, BAY- inhibitor of NFKB.

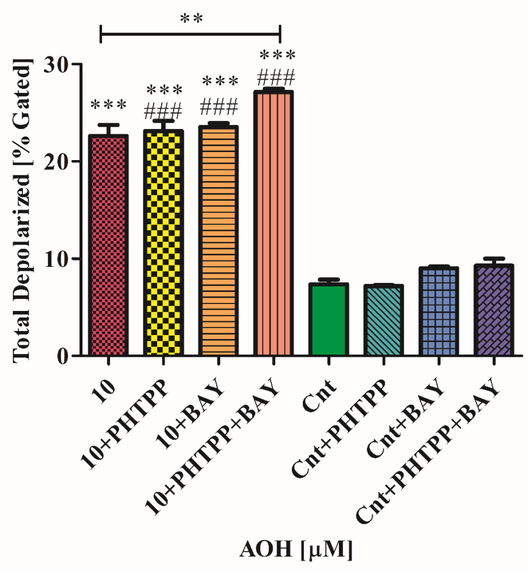


Figure 2. Alternariol induces in polarization of mitochondria. Analysis of mitochondria potential after 24 hours in SKOV3. The results are expressed as mean ± SE. One-way ANOVA with the Bonferroni post hoc test was used to calculate statistical significance. *P* value lower than 0.05 was considered statistically significant. ***p*<0.01, ****p*<0.001 as compared to control cells. ##p<0.01, ###p<0.001 as compared to adequate positive control. AOH- alternariol, Cnt- control, PHTPP- selective ERβ inhibitor, BAY- inhibitor of NFKB.

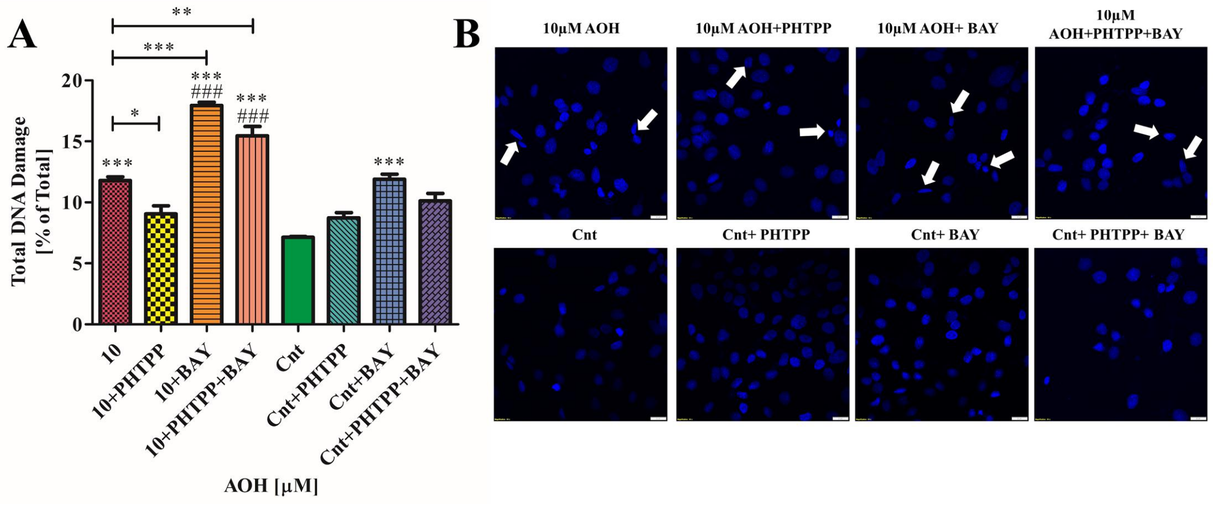


Figure 3. Alternariol induces DNA damage in ovarian cancer cells. A- The results from flow cytometry for DNA damage in the SKOV3 cells. B- DAPI staining of SKOV3 nuclei. The results are expressed as mean ± SE. One-way ANOVA with the Bonferroni post hoc test was used to calculate statistical significance. *P* value lower than 0.05 was considered statistically significant. **p*<0.05, ***p*<0.01, ****p*<0.001 as compared to control cells. ###*p*<0.001 as compared to adequate positive control. AOH- alternariol, Cnt- control, PHTPP- selective ERβ inhibitor, BAY- inhibitor of NFKB.

Conclusions:

Our results may suggest that AOH, present in our every day diet, may induce cytotoxcity in cells dependent on ERβ and NFKB