24th Scientific Symposium of the Austrian Pharmacological Society Graz, 27–28 September 2018

MEETING ABSTRACT

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12-Oxo-chenodeoxycholic acid potentiates doxorubicin-induced oxidative stress through Nrf2 axis in breast adenocarcinoma cells
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Background: As a transcription factor, nuclear factor E2-like factor 2 (Nrf2) controls the expression of genes encoding cytoprotective proteins, including antioxidant enzymes counteracting oxidative and electrophilic stress to maintain redox homeostasis. Aberrant activation of Nrf2 in malignant cells promotes high expression of cytoprotective proteins, which can decrease the efficacy of antineoplastic agents used for chemotherapy. The aim of this study was to analyse the expression of NRF2 gene as well as antioxidative system genes in a human breast adenocarcinoma cell line (MCF-7) treated with doxorubicin and the bile acid 12-oxo-chenodeoxycholic acid (12-monoketocholic acid, 12MKC).

Methods: The MCF-7 cell line was maintained in required micro-environmental conditions until confluence was reached. Cells were afterwards treated with 0.25 μM of doxorubicin (D group) or co-treated with 0.25 μM doxorubicin and 25 μM 12MKC (DM group). Following 24 h of incubation, cells were collected, RNA was isolated and transcribed into cDNA. The expression of the genes for Nrf2 (NRF2), superoxide dismutase (SOD), catalase (CAT), and β-actin (ACTB) as a housekeeping gene, was determined using RT-qPCR. Gene expression was analysed using comparative 2^-ΔΔCt method and statistical analysis was performed using Anova and Tukey’s post-hoc test.

Results: Compared to untreated group of cells, treatment of MCF-7 cells reduced expression of NRF2 both in the D and in the DM group: 2.74 ± 0.57 (p < 0.001) and 1.74 ± 0.59 (p = 0.014), respectively. Expression of SOD was also repressed in the D and in the DM group: 3.68 ± 0.78 (p < 0.001) and 1.11 ± 0.37 (p < 0.001), respectively. On the other hand, the expression of CAT was induced in the D group 1.50 ± 0.34-fold (p > 0.05), whereas suppressed in the DM group 1.15 ± 0.39-fold (p > 0.05), compared to control.

Discussion: Oncogene-induced mutations with gain of function of Nrf2 promote both ROS detoxification and tumorigenesis, whereas suppression of Nrf2 in neoplastic cells and alters redox homeostasis of malignant cells. Through suppression of NRF2, SOD and CAT genes, 12MKC exerts potential to impinge cellular antioxidative defence at the transcriptional level in MCF-7 cells treated with doxorubicin, with potential favourable effects in terms of therapeutic outcome.

Acknowledgements: Supported by Horizon 2020 MEDLEM (project no. 690876), the Provincial Secretariat for Science and Technological Development, Autonomous Province of Vojvodina (project no.114-451-2072-2016-02) and the Ministry of Education, Science and Technological Development, Republic of Serbia (grant III41012).

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