

D-homoestrone exhibits apoptosis and cell cycle arrest at G2/M in HeLa human cervical cancer cellsR Minorics¹, N Bózsity¹, E Mernyák^{0,2}, J Wölfling^{0,2}, Gy Schneider^{0,2}, I Zupkó¹¹University of Szeged, Pharmacodynamics and Biopharmacy, 6720, Hungary, ²University of Szeged, Organic Chemistry, 6720, Hungary

Although 17 β -estradiol has been described as a proliferation-inducing compound, recent experimental results indicated that certain of its analogs can exert potent antiproliferative effects without appreciable estrogenic activity. One of the most widely investigated compounds is 2-methoxyestradiol, an A-ring-modified metabolite of 17 β -estradiol via estrone formation. Its remarkable antiproliferative effect has been proved in several cancer cell lines, especially those of reproductive origin (e.g. cervical and endometrial cancer, etc.). Similarly, it is able to induce apoptosis in different cell lines. Moreover, 2-methoxyestradiol was reported to develop cell cycle arrest at G2/M through activation of p53 and checkpoint kinase-1 (Chk1) in human endometrial cancer cells.

The metabolism of estrone can continue by hydroxylation of the D-ring and various types of D-ring-modified estrone analogs have been synthesized and tested for possible inhibitory effects on cell proliferation. Our newly synthesized and until now not investigated test compound, D-homoestrone, is a D-ring expanded analog of estrone with no significant estrogenic activity neither *in vitro*, nor *in vivo*. It inhibits the proliferation of HeLa cells at 5.5 μ M concentration and after 72 h incubation the cells show the sign of programmed cell death according to the results of flow cytometry analysis and Hoechst 33258-propidium iodide double staining.

Our aim was to gain further insight into the mechanism of action of D-homoestrone on HeLa cells concerning its effect on the mode of apoptosis induction and its influence on cell cycle regulation process.

To detect the apoptosis induction the activation of caspase-3 and -9 was measured quantitatively with the help of colorimetric caspase assay kits. Measurements were performed according to the manufacturers' instructions (Sigma-Aldrich and Invitrogen, respectively). Cell cycle analysis was performed on HeLa cells treated with 2.5 and 5.0 μ M D-homoestrone for 48 h (Berényi et al., 2012, J Enzyme Inhib Med Chem). The expression of regulatory factors of G2/M cell cycle checkpoint was detected at mRNA level by reverse-transcription PCR using the corresponding primers (Berényi et al., 2012, J Enzyme Inhib Med Chem). For statistical evaluation, data were analyzed by one-way ANOVA with the Neumann-Keuls post test, using Prism 4 software.

The incubation of HeLa cells with our test compound for 72 h resulted in significantly increased activation of caspase-3 (control: 0.21 ± 0.002 unit and 5.0 μ M: 1.05 ± 0.009 unit; $p > 0.001$) and -9 kinases (control: 0.99 ± 0.2 unit and 5.0 μ M: 1.45 ± 0.1 unit; $p > 0.05$). Flow cytometry analysis exhibited a substantial increase in G2/M phase after 48 h incubation (control: $14.47 \% \pm 1.35 \%$ and 5.0 μ M: $19.61 \% \pm 1.14 \%$; $p > 0.001$). D-homoestrone significantly decreased the mRNA expression of the most G2/M checkpoint regulatory factors compared to control samples (e.g. cyclin B2 (by 23%), cdc25B (by 14%), Chk2 (by 24%), p53 (by 30%) and p21 (by 35%)), however it showed no action on the mRNA expression of cyclin-dependent kinase 1 and cyclin B1.

Our test compound induced cell cycle arrest in the G2/M phase resulted by the complex alteration in the mRNA expression of G2/M checkpoint regulatory factors. Moreover, D-homoestrone exhibited growth inhibitory effects on HeLa cells due to the induction of the intrinsic apoptotic pathway.

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