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Introduction & Objectives: Alsevirone {2'-[[[(E)-3 β -hydroxyandrost-5-en-17-ylidene]methyl]-4',5'-dihydro-1',3'-oxazole} is a new CYP17A1 inhibitor. The aim of this study was to evaluate cytotoxic effect of Alsevirone against prostate cancer cell lines and expression of apoptotic proteins.

Materials & Methods: CYP17A1 (17 α -hydroxylase and 17/20-lyase) inhibition was evaluated using microsomal fraction of testicles of rabbit. Cytotoxic effect was studied after 72 h co-incubation of prostate cancer cells (PC3, LNCaP, DU-145 and 22RV1) by MTT Assay. Alsevirone and abiraterone-induced apoptosis was studied in DU-145 cells by Muse Cell Analyzer and special Muse Detection Kits (EMD Millipore Corp., USA). Apoptosis signalling pathways were studied after incubation of DU-145 cells with 100 μ g/ml alsevirone and abiraterone. Annexin V (+) live cells were interpreted as early apoptotic cells; Annexin V (+) dead cells were identified as late apoptotic cells.

Results: 5 μ M of alsevirone inhibited the CYP17A1 activity for 56.1%. Calculated IC₅₀ for alsevirone and abiraterone: 40.2 vs 28.4 μ g/ml in PC3; 19.8 vs 36.2 in DU-145; 11.1 vs 26.5 in 22RV1; and 5.14 vs 7.6 in LNCaP.

Statistically significant increase of Annexin V(+) total apoptotic cells was found after co-incubation with both agents. In contrast with control, 100 μ g/ml alsevirone and abiraterone increased per cent of early apoptotic cells to 36.5 \pm 0.5% (p=0,001) and 17.5 \pm 1.9% (p=0,047). Increase of late apoptotic cells was detected after incubation with 100 μ g/ml alsevirone and abiraterone: from 5.26% in control to 24.09% and 12.87% respectively. Caspase 3/7 activation was demonstrated: caspase 3/7 (+) cells increased from 13 \pm 0.7% in control to 37 \pm 0.4% (p<0.001) and 34 \pm 0.1% (p<0.001) respectively. Also elevation of kinase activity was noticed: concentration of MAPK (+) cells increased from 3 \pm 0.4% in control to 9 \pm 0.4% (p=0.004) and 15 \pm 2.9% (p=0.033) after incubation with alsevirone and abiraterone respectively. At the same time concentration of Bcl-2 (+) cells decreased: from 93 \pm 1.1% in control to 85 \pm 0.9% (p=0.018) and 80 \pm 1.0% (p=0.007) after incubation with alsevirone and abiraterone respectively. Expression of phosphorylated H2A.X histone, which is considered as marker of DNA damage and of double-strand breaks, changed in Alsevirone-treated cells only. Alsevirone led to increase of H2A.X (+) cells count: 55 \pm 2.3% (p=0.001) in contrast with 2 \pm 1.1% in control and 2 \pm 0.1% (p=0.754) induced by abiraterone.

Conclusions: New CYP17A1 inhibitors demonstrated significant cytotoxic activity in vitro, reflected in activation of Annexin V-associated apoptosis in cancer cells. Cytotoxic activity of alsevirone was higher than of abiraterone. Apoptosis in prostate cancer cells was associated with activation of caspase 3/7, MAPK and reduction of intracellular Bcl-2. Alsevirone, in contrast to abiraterone, may influence the pH2A.X histone expression.

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