P26

In vitro cell responses and cell-cell interactions upon xenobiotic exposure of renal and hepatic cells

M. Sramkova*, E. Celkova, L. Balintova, & M. Mesarosova

Biomedical Research Center SAS, Bratislava, Slovakia
** monika.sramkova@savba.sk*

Traditionally, *in vitro* test systems are based on two-dimensional (2D) cell cultures, which are associated with inherent limitations. Lately, 3D models (spheroids) have also proven to be a very useful and promising tool in environmental toxicology, including long-term repeat dose studies enabling the exposure to lower concentrations of pollutants that are relevant also for real human exposure.

Two human cell lines, hepatocarcinoma cells (HepG2) and renal proximal tubule epithelial cells (TH1) were used to evaluate the biological activity of two chosen xenobiotics, aflatoxin B1 (AFB1) - a potent genotoxic hepatocarcinogen and ifosfamide (IFO) - a synthetic analog of cyclophosphamide that has a nephrotoxic effect, in various *in vitro* test systems (2D – monolayer, 3D – spheroids, co-cultures).

The objective of this study was to evaluate the cytotoxic and genotoxic effects after 2h and 24h exposure to AFB1 and IFO, the changes in ROS production, and the expression of enzymes involved in the metabolism of xenobiotics, especially the P450 cytochrome complex.

The evaluation has shown that in 2D models, short-term as well as long-term exposure to IFO decreased the cell viability of both TH1 and HepG2 cells, while the cytotoxic effect of AFB1 was detected only after long-term exposure in both cell lines. The genotoxic effect was determined by comet assay and micronucleus test. AFB1 and also IFO were able significantly to increase the level of DNA strand breaks in both in vitro systems, while the most damaging was 24h IFO exposure. Chosen xenobiotics significantly increased the percentage of micronuclei in both cell lines after 2h and 24h treatment.

In 2D after 24h cell exposure, we have also measured the low significant increase in ROS production. The effect of both chemicals has also caused changes in CYPs expression (CYP1A2, CYP2B6, CYP3A4) in both cell systems which was determined by western blotting.

As expected, our results showed different cell responses upon AFB1 and IFO treatment, confirming the differences between cell lines along with the culture conditions. This study was supported by VEGA grant 2/0121/21, project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381, and APVV 20-0494.

Keywords:
Toxicity, xenobiotics, 3D cultures, kidneys, liver