Toxicity assessment of xenobiotics: A comparison of 2D and 3D in vitro models

Monika Mesarosova, Lucia Balintova, Monika Sramkova

Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovakia
monika.mesarosova@savba.sk

Animal models and conventional two-dimensional (2D) cell culture models have long been used to understand animal and human physiology and pathology. Cell-based assays play an important role in the drug development process and safety assessment of chemicals and drugs as a fast, cost-effective, and straightforward approach to reduce animal testing. 2D *in vitro* test systems based on monolayer cultures are associated with inherent limitations. Therefore, it is essential to develop alternative *in vitro* cell-based systems which offer an alternative platform for predicting the efficacy, toxicity, and pharmacokinetics of new drugs and can represent an innovative approach to screening for xenobiotic effects and reducing animal testing. 3D models (spheroids) have also proven to be very useful and promising tool.
In vivo, the liver and the kidney are the major organs in which xenobiotics are metabolized, transformed and play a key role in detoxification and their elimination from the body. They also represent two primary targets of the toxic effect of xenobiotics.

Two human cell lines, hepatocyte carcinoma 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 model – HepG2 spheroids).
The objective of this study was to evaluate the cytotoxic and genotoxic effect after short-term (2h) as well as long-term (24h) cell exposure to xenobiotics (AFB1 and IFO). The cytotoxic effect was determined by MTT assay in 2D models (TH1 and HepG2) and by LDH assay in 3D spheroids (HepG2). The evaluation has shown that in 2D models 2h and also 24h exposure to IFO decreased the cell viability of both TH1 and HepG2, while the cytotoxic effect of AFB1 was detected only after long-term exposure in both cell lines. In 3D spheroids, the cellular cytotoxicity was measured only after 24h exposure to IFO.

The genotoxic effect was determined by comet assay (2D, 3D) and micronucleus analysis (2D). 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 the long-term IFO exposure. Chosen xenobiotics significantly increased the percentage of micronuclei in both cell lines after 2h and 24h treatment in 2D cell systems.
Consequently, we will also determine the changes in the expression of enzymes involved in the metabolism of xenobiotics, especially the P450 cytochrome complex.

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 is based upon work supported by VEGA grant 2/0121/21, and project VISION (Strategies to strengthen scientific excellence and innovation capacity for early diagnosis of gastrointestinal cancers) No. 857381.