Use of advanced *in vitro* systems for risk assessment of xenobiotics

Eva Celkova, Lucia Balintova, Monika Mesarosova, Monika Sramkova

Cancer Research Institute, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

The effort to reduce the use of animal models in preclinical and toxicity tests is closely linked to the development of innovative test systems that will allow better prediction of the *in vivo* response.
In vivo, the liver is the major organ in which xenobiotics are metabolized and transformed, and the kidneys are subsequently responsible for their uptake, concentration, and elimination from the body. Thus, both organs play a key role in the detoxification and elimination of xenobiotics and their metabolites and represent the two primary targets of the toxic effect of xenobiotics.
The studied xenobiotics were chosen based on their effect: aflatoxin B1 (AFB1) - a very potent genotoxic hepatocarcinogen, ifosfamide (IFO) - a synthetic analog of cyclophosphamide that has a nephrotoxic effect. The cytotoxic and genotoxic effects of AFB1 and IFO were studied on stable human cell lines HepG2 (liver) and TH-1 (kidney), in various *in vitro* conditions (monolayer, 3D spheroids, co-culture).
Our data showed that IFO had a stronger cytotoxic effect after 2 h in TH-1 cells than HepG2 cells (IC50 35 mM vs. 75 mM in HepG2). After 24 h, IC50 was reached at 25 mM for both cell lines. AFB1 did not show cytotoxicity after 2 h using selected concentrations (0.5-100 μM), after 24 h HepG2 cells showed higher sensitivity (IC50 60 μM vs. 100 μM in TH-1 cells). Differences in IC50 were observed in different culture conditions (spheroids, co-culture). Both chemicals were able to significantly increase the level of DNA breaks as detected by comet assay and the percentage of micronuclei in both cell lines cultivated in monolayers.
As expected, our results showed different cell responses upon AFB1 and IFO treatment, confirming the differences between cell lines along with the culture conditions.

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