Comparative genotoxicity of *Alternaria* mycotoxins in HepG2 cells

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Background: *Alternaria* mycotoxins are widespread emerging food contaminants to which humans are frequently exposed through diet. However, toxicological data on several relevant *Alternaria* toxins remain limited, particularly regarding their potential hepatic genotoxicity.

Aims: This study aimed to evaluate the cytotoxic and genotoxic effects of six selected *Alternaria* mycotoxins — alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), altertoxin-I (ATX-I), altenuene (ALT), and tentoxin (TEN) — in human hepatocellular carcinoma (HepG2) cells.

Methods: Exponentially growing cells were exposed to mycotoxins for 1.5-2 cell cycles lenght (48 h – 51 h). Cytotoxicity was firstly assessed by the MTT assay and then using the cytokinesis-block proliferation index (CBPI) to define the concentration-range for genotoxicity testing. The cytokinesis-block micronucleus (CBMN) assay (OECD Test Guideline 487) was selected for genotoxicity assessment.

Results: A differential toxicity profile was observed among the tested mycotoxins (MTT assay), with AME showing the highest cytotoxicity (IC_{50} = 20.06 µM), followed by ATX-I (IC_{50} = 56.84 µM), AOH (IC_{50} = 116.78 µM) and TeA (IC_{50} = 446.31 µM). In contrast, both ALT and TEN showed limited cytotoxicity (< 20%) under the tested conditions. All six mycotoxins induced a significant increase in the frequency of micronucleated cells at least at one tested concentration, compared with the vehicle control, showing their ability to cause chromosomal damage in HepG2 cells. ATX-I and AOH were the most potent genotoxic compounds, inducing significant micronucleus formation from 0.25 µM and 6.25 µM onward, respectively. AME, ALT, TEN, and TeA induced significant genotoxic effects from 20, 25, and 50 µM onward, respectively.

Conclusion: Overall, these findings indicate that *Alternaria* mycotoxins can exert genotoxic effects in hepatic cells, suggesting a potential ability to induce carcinogenic processes. These results provide relevant evidence for the hazard and risk assessment of these mycotoxins, contributing to their future regulation.

Acknowledgements: Doris Marko (University of Vienna, Austria) and Ann-Cathrin Behr (Bundesinstitut für Risikobewertung, BfR, Berlin, Germany).

Funding: Work funded by the European Union's Horizon Europe research and innovation programme under Grant Agreement No.101057014 (PARC) and co-funded by Instituto Nacional de Saúde Dr. Ricardo Jorge.