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DNA replication stress and an ATR-HIPK2 signaling branch mediate genotoxicity of the mycotoxin Aflatoxin B1

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Mycotoxins, including Aflatoxin B1 (AFB1), pose major medical problems since they can contaminate human and pet food. Mycotoxin incorporation is linked to acute and chronic toxicity by causing acute liver damage and inflammation, while chronic exposure is linked to liver carcinogenesis. Although it is well established that AFB1 causes bulky, mutagenic DNA base adducts, the detailed mechanisms and DNA damage signalling events underlying acute AFB1 cytotoxicity are still incompletely defined. The DNA damage-activated kinase HIPK2 is a central regulator of cell death in response to genotoxic stress. The role of HIPK2 in mycotoxin toxicity remains currently unknown.

Here we analysed the molecular events and DNA signalling pathways underlying acute mycotoxin genotoxicity. Our results revealed a critical role for HIPK2 in AFB1-mediated genotoxicity. Acute exposure of human liver cells to AFB1 results in site-specific HIPK2 autophosphorylation and activation. In consequence, active HIPK2 phosphorylates p53 at Ser46 and triggers cell death through both ferroptosis and apoptosis induction. Pharmacological HIPK2 inhibition inhibits p53 Ser46 phosphorylation. Furthermore, we found that AFB1 exposure triggers DNA replication stress involving checkpoint kinase ATR activation, RPA phosphorylation and stalling of replication forks, reflected by DNA fibre assays. In addition, by making use of select kinase inhibitors we demonstrate that AFB1-induced HIPK2 activation is mediated by checkpoint kinases ATR thereby linking HIPK2 activation to DNA replication stress. Confocal microscopy revealed that upon AFB1 exposure active HIPK2 accumulates at DNA breaks in an ATR-dependent manner, suggesting HIPK2 activation takes place at DNA breaks. Pharmacological inhibition of ATR and the down-stream activated checkpoint kinase ATM inhibits AFB1 cytotoxicity in liver cells, suggesting DNA damage checkpoint kinase inhibition as potential intervention option to mitigate acute AFB1 hepatotoxicity. Collectively, our results reveal DNA replication stress as a critical mechanism of AFB1 hepatotoxicity through triggering a detrimental ATR-HIPK2-p53 signalling axis. Finally, our data suggest select DNA damage checkpoint kinase inhibitors as potential antidots to treat acute mycotoxin toxicity.

Keywords:
mycotoxins; Aflatoxin B1; DNA replication stress; ATR; HIPK2.