

Biotransformation and Lack of Mutagenicity of Ochratoxin A Using Combinations of Mammalian Biotransformation Enzymes

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SUMMARY

The genotoxicity of ochratoxin A was studied in many in vitro systems, mostly negative or inconclusive results were obtained. However, in recent studies, a mutagenic response was observed in mammalian cells transfected with certain human cytochrome P450 enzymes. Moreover, an involvement of glutathione in this mutagenic response is indicated by a reduced mutagenicity after glutathione depletion. Ochratoxin A was incubated with cytochrome P450 containing protein fractions for varying times. Formation of metabolites was assessed by HPLC with UV- and fluorescence detection. Enzymatic activity of specific P450 enzymes in the fractions was determined using established marker substrates. For some incubations, subcellular fractions with glutathione S-transferase activity and glutathione were added to the incubations. Using rat liver cytochromes P450, biotransformation of ochratoxin A to metabolites was observed by cytochrome P450 1A1/2 and 3A. Cytochrome P450 3A likely results in hydroxylation of the isocoumarin ring, P450 1A1/2 forms an ochratoxin A metabolite in low yields which has not been characterized. Addition of subcellular fractions with glutathione S-transferase activity and glutathione did not modify the products formed based on HPLC retention and UV- or fluorescence spectra. Incubations with genetically expressed human P450 3A4, 1A1/2, 2C9 and ochratoxin A resulted in product formation in very low yields despite a high activity of the preparation with established marker substrates. Ochratoxin A mutagenicity was studied in the presence of human cytochromes P450 and purified glutathione S-transferases. No mutagenic response was observed. The experimental results obtained to date indicate that ochratoxin A is a poor substrate for cytochromes P450s and glutathione dependent biotransformation reactions are unlikely to occur due to the lack of formation of electrophilic intermediates. Based on these results, formation of DNA-binding of intermediates in ochratoxin A metabolism seems unlikely.