In Vitro Cytogenetic Results Supporting a DNA Nonreactive Mechanism for Ochratoxin A, Potentially Relevant for Its Carcinogenicity

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Ochratoxin A (OTA) is a widespread mycotoxin of cereals and many agricultural products and causes high incidences of renal tumors in rodents. Although its carcinogenic properties have been known since the eighties, the precise mechanism of action is still relatively undefined. At present, increasing evidence suggests that OTA does not act with a direct genotoxic mechanism, opposed to other previous evidence where the formation of DNA adducts by ³²P-postlabeling was observed. The genotoxic activity of OTA assessed in a variety of in vitro and in vivo studies was very low if genotoxic at all. In this study, we clearly show that OTA does not bear any clastogenic or aneugenic activity based on the absence of the induction of chromosome aberrations, sister chromatid exchanges, and micronuclei in human lymphocytes and V79 cells in vitro in both the absence and the presence of S9 metabolism. Alternatively, cytogenetic analyses evidenced significant increases in endoreduplicated cells and highly condensed metaphases with separated chromatids. This implies that OTA or its possible metabolites do not covalently bind DNA through the formation of adducts since structural chromosome aberrations are a very sensitive end points to detect chemical carcinogens with electrophilic substituents. Alternatively, induction of endoreduplication and chromatid separation provides strong evidence for a DNA nonreactive mechanism of OTA carcinogenicity involving the disruption of mitosis by interfering with key regulators of chromosome separation and progression of mitosis. This causes a temporary arrest of mitoses and premature exit from it (mitotic slippage) to generate endoreduplication and polyploidy accompanied by increased risk of aneuploidy and subsequent tumor formation.

Introduction

Ochratoxin A (OTA)¹ is a mycotoxin produced by several species of fungi (*Penicillium* and *Aspergillum* species), and it occurs naturally in cereal and grain products. OTA is a nephrotoxic mycotoxin with strong carcinogenic properties to rodents (1, 2) and possesses teratogenic, immunotoxic (3–5), and possibly neurotoxic activities (6, 7). Furthermore, it has been considered potentially responsible for the development of nephropathies observed in different farm animals (8). In humans, it is supposed to be associated with Balkan endemic nephropathy (BEN) and is possibly involved in the origin of urinary tract tumors (9). On these bases, OTA has been classified as a possible human carcinogen by the International Agency for Research on Cancer (IARC) (10). Since then, the carcinogenic mechanism of OTA has still been relatively undefined, although at present increasing evidence suggests that OTA does not act with a direct genotoxic mechanism. This is supported by different studies with radiolabeled OTA unable to detect any

DNA binding of OTA (11–14) as opposed to previous studies using the ³²P-postlabeling method in which DNA adducts were reported (15–17), although the authors did not provide structural information on the nature of the adduct. On the other hand, the genotoxic activity of OTA assessed in a variety of in vitro and in vivo studies was very low if genotoxic at all. OTA did not prove to be mutagenic in *Salmonella typhimurium* in both the absence and the presence of metabolic activation systems, when assays were conducted according to standard protocols (2, 18–22), although mutations after exposure to OTA were reported using nonvalidated modifications of the Ames test (23). OTA was also negative in a *Salmonella* microsome assay with a HepG2-derived enzyme (S9 mix) with the TA98 and TA100 strains (24) and in the *Escherichia coli* SOS spot test at nontoxic concentrations (25–27). In mammalian cells, conflicting results were obtained regarding the potential of OTA to induce mutations. OTA was not mutagenic in thymidine kinase (TK±) mouse lymphoma cells or in the hypoxanthine–guanine phosphoribosyltransferase (HPRT) test system (19, 22). In contrast, mutagenic effects of OTA were reported in NIH 3T3 mouse fibroblasts stably transfected with human cytochrome P450s (28), although these results are difficult to interpret since studies on OTA metabolism indicate that bioactivation does not play a role in OTA toxicity and carcinogenicity (11–13, 29, 30). In Chinese hamster ovary (CHO) cells, chromosomal aberrations were not seen after exposure to OTA, but only a small increase in the frequency of sister chromatid exchanges (SCEs) was noted

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¹ Abbreviations: OTA, ochratoxin A; SCEs, sister chromatid exchanges; CHO, Chinese hamster ovary; PHA, phytohemagglutinin; BrdU, 5-bromo-2'-deoxyuridine; MI, mitotic index; CBPI, cytokinesis-block proliferation index.