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Estrogenic and clastogenic potential of the mycotoxin alternariol in cultured mammalian cells

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Abstract

The mycotoxin alternariol (AOH) is found in food and beverages infected by *Alternaria alternata.* Because consumption of foodstuffs contaminated with *A. alternata* has been implicated in an elevated incidence of esophageal carcinogenesis, we have investigated the estrogenic potential, the effect on cell proliferation, and the genotoxic effect of AOH in cultured mammalian cells.

AOH replaced E2 from isolated human estrogen receptors α and β and increased the level of alkaline phosphatase (ALP) mRNA and the enzymatic activity of ALP in a human endometrial adenocarcinoma cell line (Ishikawa cells). The estrogenicity of AOH was about 0.01% of that of E2. The effects in Ishikawa cells were reversed by the ER antagonist ICI 182,780.

Analysis of cell proliferation by flow cytometry and microscopy of Ishikawa and Chinese hamster V79 cells revealed that AOH inhibited cell proliferation by interference with the cell cycle.

The genotoxic potential was assessed by the micronucleus (MN) assay and immunochemical differentiation between MN containing whole chromosomes (kinetochore-positive) and DNA fragments (kinetochore-negative) in Ishikawa and V79 cells. AOH induced kinetochore-negative MN in both cell lines.

This is the first report on the estrogenic potential, inhibition of cell proliferation and clastogenicity of AOH in Ishikawa and V79 cells in vitro.

Keywords: Alternariol; Micronuclei; Estrogen; Ishikawa cells; V79 cells

1. Introduction

The level of contamination with *Alternaria alternata* in corn from areas of high morbidity from esophageal cancer is higher than that in low-morbidity areas (Liu et al., 1992). Therefore, consumption of foodstuffs contaminated with *A. alternata* has been implicated in an elevated incidence of esophageal carcinogenesis (see Liu et al., 1991 and literature cited therein). In support of this association, it has been shown that extracts of *A. alternata* are toxic, genotoxic and mutagenic in vitro and carcinogenic in experimental animals: extracts of *A. alternata* were toxic to HeLa cells and laboratory animals (Ohtsubo et al., 1978; Sauer et al., 1978; Younis and Al-Rawi, 1988) and induced reverse mutations in *Escherichia coli* and *Salmonella typhimurium* strains TA98 and TA100 (Scott and Stoltz,

Abbreviations: ALP, alkaline phosphatase; AOH, alternariol, 3,4′,5-trihydroxy-6′-methyldibenzo-pyrone; BPA, bishenol A; BSA, bovine serum albumin; CMTC, cytoplasmic microtubule complex; CREST, calcinosis, Reynaud's phenomenon, esophageal mobility abnormalities, sclerodactyly and telangiectasia; DAPI, 4,4′-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; E2, 17β-estradiol; EDTA, ethylenediaminetetraacetic acid; ER, estrogen receptor; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HEPES, *N*-(2-Hydroxyethyl)piperazine-*N*′-ethanesulfonic acid; HPRT, hypoxanthineguanine phosphoribosyltransferase; MN, micronucleus; PBS-CMF, phosphate-buffered saline free of calcium and magnesium; PCR, polymerase chain reaction; PMSF, phenylmethanesulfonylfluorid; RT-RCR, reverse transcription PCR; TAE, tris/acetic acid/EDTA; Taq, thermophilus aquaticus; UV, ultraviolet.

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1980; Schrader et al., 2001), unscheduled DNA synthesis in cultured human amniotic cells (Liu et al., 1991 and literature cited therein) chromosomal aberrations and sister chromatid exchanges in human peripheral blood lymphocytes (Chen et al., 1985; Dong et al., 1987a), mutations in V79 cells and transformations of NIH3T3 cells (Dong et al., 1987b; Dong et al., 1988). Food mildewed by *A. alternata* induced forestomach tumors in rats (Liu et al., 1992).

Despite the established mutagenicity of *A. alternata* extracts, little is known about the biological effects of the individual mycotoxins produced by this mould. Alternariol (AOH, Fig. 1) is one of the main mycotoxins formed in foods and feeds infected by *A. alternata*. Occurrence of AOH has been reported in various fruits, including tomatoes, olives, mandarins, melons, peppers, apples, and raspberries; it has also been found in processed fruit products such as apple juice, other fruit beverages and tomato products, wheat and other grains, sunflower seeds, oilseed rape meal, and pecans (Scott, 2001). Moreover, AOH methylether (the other major metabolite of *A. alternata*) can be demethylated metabolically by liver homogenate of gilts to AOH (Olsen and Visconti, 1988).

No evidence of toxicity was observed in rats or chickens when AOH was fed for 21 days in doses approximately 6-fold higher than those found in heavily mildewed sorghum grain at harvest (Sauer et al., 1978). Previously, it has been reported that AOH competitively inhibited acetylcholinesterase of rat brain in vitro (Mohammed et al., 1974), induced lipid peroxidation in the epithelium of fetal esophagus in vitro (Liu et al., 1992) was phototoxic, DNA-intercalating, and DNA cross-linking to *E. coli* in the presence of UV light (DiCosmo and Straus, 1985), and caused DNA single-strand breaks in closed circular double-stranded supercoiled DNA (Xu et al., 1996) and cultured primary rat hepatocytes (Liu et al., 1992). However, the mutagenicity of AOH in bacterial in vitro systems is still unclear. Whereas one study showed that AOH was nonmutagenic to strains TA98 and TA100 with and without metabolic activation (Davis and Stack, 1994), another laboratory found AOH to be weakly mutagenic in the presence and absence of metabolic activation in TA100, and responses obtained with metabolic activation were enhanced by nitrosylation (Schrader et al., 2001).

The few reports on the genotoxic activity of AOH in mammalian systems showed the formation of DNA adducts by AOH in FL cells (Wang et al., 1994) as well as an amplification of the epidermal growth factor receptor gene (Shan et al., 2000) and mutations at codon 12 of the cellular ras gene (Dong et al., 1993) in human fetal esophageal epithelium in vitro.

Another possible activity of AOH not studied at all to date is its estrogenicity. AOH is a diphenolic compound with a chemical structure similar to certain natural and synthetic estrogen-mimicking substances such as resveratrol, genistein, and bisphenol A.

The aim of the present study was to examine the biological activity of AOH at three important in vitro endpoints: (i) the estrogenic activity, (ii) the effect on cell proliferation, and (iii) the genotoxic potential. The estrogenic potential was investigated by competitive replacement of radiolabeled 17β-estradiol (E2, Fig. 1) from the recombinant human estrogen receptors (ER) α and β in vitro, and the influence of AOH on the ER-dependent expression of the alkaline phosphatase (ALP) gene in a human endometrial adenocarcinoma cell line (Ishikawa cells). Effects on cell proliferation were determined by flowcytometric analysis of the number of nuclei and the cell cycle distribution and fluorescence microscopic analysis of Ishikawa and Chinese hamster V79 cells, and the genotoxic potential was assessed by the micronucleus (MN) assay in Ishikawa and V79 cells.

We report here for the first time that AOH exhibits estrogenic potential in vitro. Furthermore, AOH inhibited cell proliferation and proved to be a strong clastogen in V79 cells.

2. Materials and methods

2.1. Chemicals

AOH (CAS No. 641-38-3) and E2 (CAS No. 50-28-2) were purchased from Sigma (Taufkirchen, Germany). The purity of AOH was >99% according to the manufacturer. All other chemicals, cell culture media and medium supplements were obtained from Sigma or Roth (Karlsruhe, Germany) if not specified otherwise.

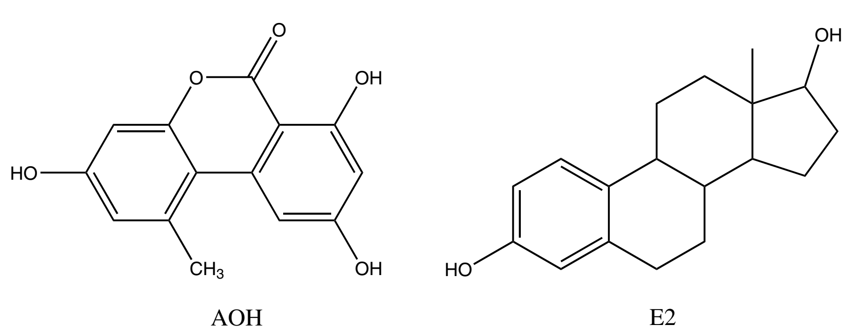

Fig. 1. Chemical structure of AOH and E2.