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SHORT RESEARCH PAPER

Allium cepa as a biomonitor of ochratoxin A toxicity and genotoxicity

D. Lerda[1], M. Biagi Bistoni[1], P. Pelliccioni[1] & N. Litterio[2]

1 Genetic Laboratory, School of Medicine, Cordoba Catholic University, Córdoba, Argentina

2 Agrarian Sciences School, Cordoba Catholic University, Córdoba, Argentina

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Correspondence

D. Lerda, Genetic Laboratory, School of Medicine, Cordoba Catholic University, Córdoba, Argentina.

E-mail: dlerda@coyspu.com.ar

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ABSTRACT

Ochratoxin A (OTA) is a toxin produced by *Aspergillus* and *Penicillum* moulds. Since OTA has not yet been evaluated in plant systems, this paper focused on describing the controversial effect OTA in an *Allium* root test model, which has known sensitivity to genotoxins and could be useful in toxin screening. Analyses of root growth and the root meristematic zone in response to OTA treatment were undertaken. The results show OTA toxicity to root growth at a concentration of 10 ug·ml^{-1} associated with inhibition of proliferation activity. Cytological changes observed in the *Allium* chromosome aberrations assay, at a concentration of 5.0 ug·ml^{-1}, showed that OTA was able to induce genotoxicity at the chromosome level. These results indicate that plants cells (*Allium cepa*) are very sensitive to the mycotoxin OTA, as observed at the highest concentration. Under these conditions, OTA produced toxicity and cytogenetic injury. Evidence *in vitro* and *in vivo* indicates that OTA can induce damage at the DNA level.

INTRODUCTION

Ochratoxin A (OTA) is a toxin produced by the mould species, *Aspergillus* and *Penicillum*. The results of currently available *in vitro* genotoxicity assays do not fully support the assumption that OTA is a genotoxin: negative results were found in DNA repair assays using primary rat hepatocytes (Bendele *et al.* 1985) and in *Salmonella* microsome assays with S9 mix (Kuczuk *et al.* 1978; Wehner *et al.* 1978; Bendele *et al.* 1985; Würgler *et al.* 1991). Evidence for a direct genotoxic effect was reported in (i) unscheduled DNA synthesis with rat hepatocytes and porcine urinary bladder epithelial cells (Dörrenhaus & Föllmann 1997); (ii) chromosomal aberrations were observed in human lymphocytes exposed to OTA (JECFA 2001); (iii) micronucleus formation in ovine seminal vesicle cell cultures (JECFA 2001); and (iv) micronucleus formation in Syrian hamster embryo fibroblasts (JECFA 2001). In recent investigations, clastogenic activity in human-derived hepatoma (Hep G2) cells was found (Ehrlich *et al.* 2002) and DNA damage and cytogenetic effects in rat cells were reported (Mally *et al.* 2005).

The common onion (*Allium cepa*) is an excellent plant for the assay of chromosome aberrations that occur after chemical treatment. Protocols were developed using root tips from either bulbs or seeds of *A. cepa* to study cytological endpoints, such as chromosome breaks and exchanges, which follow the testing of chemicals in somatic cells. In this background, and since OTA has not yet been evaluated in plant systems, it was of interest to study toxicological aspects of OTA in relation to plant cells. The aim of the present study was to investigate the genotoxic effects of OTA in *A. cepa*. Since OTA has not yet been evaluated in plants systems, the research focused on describing the controversial effects that OTA when *A. cepa* is used as a vegetal model to demonstrate sensitivity in genotoxin screening.

MATERIALS AND METHODS

Bulbs and adventitious roots

Onion bulbs of the Pearl variety, average weight 20 g, were used. Adventitious roots were obtained by placing the base of bulbs in filtered water in glass tubes equipped with a constant air bubbling system (10–20 ml·min^{-1}) in an incubator at 25 ± 0.5 °C in darkness. Control bulbs were incubated in filtered water. Pure, crystalline OTA solutions (Sigma Chemical Co, St Louis, MO, USA) were prepared at concentrations of 0.25, 0.50, 1.0, 2.0, 5.0 and 10 μg·ml^{-1} in (DMSO) and diluted with filtered water. Water was used as the negative control. In order to determine the concentrations necessary for the genotoxicity test, root growth was monitored over 72 h. Between 20 and 30 roots were used at each concentration of OTA, and the average of root lengths were measured and used for calculation of percentage growth of exposed roots with respect to the control. These values were used to produce a dose–response curve.

The OTA concentration of 10 μg·ml^{-1} was eliminated from the genotoxicity analysis (Fiskesjo 1985). All preparations were administered when roots were 2–3-cm long.

Root growth measurement

Root growth was determined by measuring the length of 10–12 previously identified roots per bulb every 24 h for 96 h.

Proliferation activity

Proliferation activity was quantified by determining mitotic cell frequency at the root tip. Roots obtained at 0, 12, 24 and 48 h after the beginning of the assay were fixed in ethanol-acetic acid (3:1 v/v) at 4 °C for 24 h. The root meristematic area was squashed after being stained with acetic-orcein. The frequency of mitotic cells was determined in 1000 squashed cells.

Chromosomal aberrations

When roots were 3–5-cm long they were exposed to the OTA concentrations for 24 h. After that, they were exposed to colchicine at 0.1% for 3 h. Roots were then cut and fixed in ethanol–acetic acid (3:1 v/v) at 4 °C for 24 h, then stained with acetic orcein. Approximately 5000 cells were scored for frequency and type of chromosome aberration. Ethyl methyl sulphoxide (EMS) at 0.2% was used as a positive control. The frequency (percentage) of aberrant cells was determined on the basis of the total number of computed cells and the number of dividing cells.

Statistics

The Irwin–Fischer (Z) assay for exact probability was used to determine significant differences between treated and control onion roots.

RESULTS

The effect of different OTA concentrations on longitudinal growth roots was analysed (Fig. 1). OTA concentrations of 10 $\mu g \cdot ml^{-1}$ arrested growth after 24 h, without apparent root death. At concentrations of 0.25, 0.50, 1.0, 2.0 and 5.0 $\mu g \cdot ml^{-1}$, root growth rate was reduced compared to control roots. These findings indicate that OTA causes inhibition of root growth in a concentration-dependent manner. In terms of cell proliferation, the frequency of mitotic cells progressively decreased with increasing OTA concentration

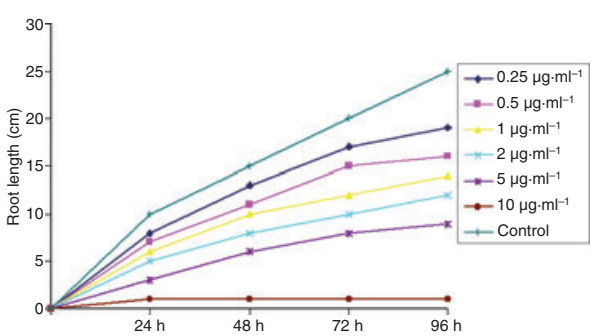

Fig. 1. Effects of OTA on onion root length.

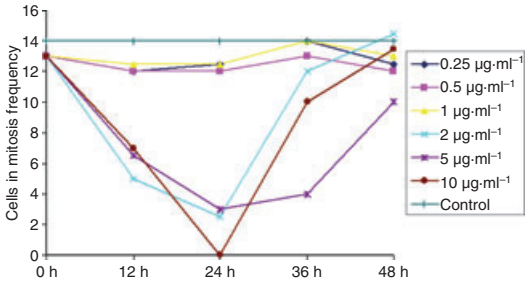

Fig. 2. Effects of OTA on proliferation activity in the meristematic zone of onion roots.

(Fig. 2), reaching a minimum after 24 h. This inhibition was transient, as recovery of proliferation activity was observed after 48 h of OTA incubation. These findings suggest that OTA blocks the cell division cycle at a stage before mitosis. Onion roots exposed to 0.25, 0.50, 1.0 and 2.0 $\mu g \cdot ml^{-1}$ OTA had similar frequency of mitotic cells to control roots. The chromosomal aberrations (CA) were studied with OTA concentrations of 0.25, 0.50, 1.0, 2.0 and 5.0 $\mu g \cdot ml^{-1}$, since 10.0 $\mu g \cdot ml^{-1}$ stopped growth after 24 h. OTA induced clastogenic effects in meristematic roots of *Allium cepa*. Data on the frequency of aberrant cells are presented in Table 1. An increase in the frequency of aberrant cells was observed with the highest OTA concentration (5.0 $\mu g \cdot ml^{-1}$). The Irwin–Fischer (Z) test for exact probability was carried out using the total number of aberrant cells, since 5.0 $\mu g \cdot ml^{-1}$ OTA only ruptured cells and produced binucleate cells that differed from those of the positive control. The Irwin–Fischer Z was 7.01 (>1.90) and significant at $P < 0.05$. The detected CA included ruptured chromatids and binucleate cells among other cell types.

DISCUSSION

Allium cepa offers a good experimental model for *in vivo* evaluation of genotoxicity of substances and complex mixtures. Although OTA toxicity has been evaluated in several experiments in *in vivo* and *in vitro* prokaryotic and eukaryotic cells (World Health Organization 2008), there are no reports of the effects of this compound in plant cells. Ours findings suggest that OTA inhibits longitudinal growth of the root as a result of the concentration applied. The toxic effect of root growth inhibition can be due to OTA interference with processes associated with root elongation in the zone contiguous to the meristem. It has been known for several decades that different *A. cepa* root zones have different electronegativity from the apex to the base. Oxygen consumption associated with respiration is higher in the meristematic zone, where cell division is most active (Berry & Brock 1946). Considering that the reduction of the OTA depends on oxide reduction conditions, as previously demonstrated, it is plausible that the reduction occurs in the elongation zone, where oxidation reactions are less favourable than in the meristematic zone. This could lead to a toxic effect of OTA and growth inhibition of the root, demonstrating that OTA acts in elongation zone and causes toxicity in the meristematic zone. Recent

Table 1. Frequency and spectrum of cytological aberrations induced by OTA in the *Allium cepa* assay system.

			abnormalities						frequency of aberrant cells (%) based on	
treatment	total number of Cells	cells in division	breaks	bridges	stickiness	disturbed meta/anaphase	binucleate cells	total aberrant cell	total cell scored	number dividing cell
Ochrataxin A										
0.25 ug·ml^{-1}	4500	359	–	–	–	–	–	–	–	–
0.50 ug·ml^{-1}	4500	360	–	–	–	–	–	–	–	–
l.0 ug·ml^{-1}	4500	370	–	–	–	–	–	–	–	–
2.0 ug·ml^{-1}	4500	344	–	–	–	–	–	–	–	–
5.0 ug·ml^{-1}	4500	370	6	–	–		5	11*	0.26	3.1
negative control	5000	366	–	–	–	–	–	–	–	–
positive control[a]	5000	361	7	8	18	7	4	44	0.99	11.0

–: No clastogenic effects.

*Z = 7.01 significant at $P < 0.05$ compared with negative and positive controls.

[a]Ethyl methyl sulfoxide (0.2%).

publications attribute to the regulation of elongation and differentiation by the root of *A. cepa* to differences in ascorbate content and enzymes related to the oxidation state, as well as to presence and activity of hydrogen peroxide and the peroxide ion. The content of these metabolites and enzymes varies in different zones of the root, depending on the degree of cellular differentiation, physiological state and cell metabolic requirements (Cordoba-Pedregosa *et al.* 2003). OTA blocks cell division at a stage before mitosis.

It was also found that onion roots possess OTA elimination or neutralization mechanisms. Based on the results observed in the *A. cepa* test, we suggest that OTA is a chemical with clastogenic activity. When analysing our results, we found a high incidence of effects of OTA on meristematic cells that had compromised morphology. These cells, when observed in an optical microscope, seemed to be unstructured, with a fragile cellular envelope, increased cell volume and, in several cases, rupture of the cellular membrane. These observations are indicative of cell death (Cristea & Esposti 2004), where an increase in cell volume leads to rupture of the plasma membrane, exposing the intracellular contents to the external environment.

Cytologic changes observed in the *Allium* chromosome aberrations assay, at concentrations of 5.0 μg·ml^{-1} OTA, showed that OTA was able to induce genotoxicity at the chromosome level. The *A. cepa* assay has excellent correlations with mammalian systems (Grant 1982). Results obtained on *Allium* Chromosomal Aberrations (CA) may be used as a relevant test for the general detection of genotoxin in the environment (Grant 1982, 1994; Fiskesjo 1985, 1988, 1993; Lerda 1992; Nielsen & Rank 1994; Smaka-Kinel *et al.* 1996; Bolle *et al.* 2004; Feretti *et al.* 2007). There are no studies reporting OTA adverse effects on plants cells. The present results indicate that plant cells (*Allium cepa*) have a very sensitive cellular response to the mycotoxin, OTA, at the highest concentration used. This concentration caused toxicity and cytogenetic injury. Evidence *in vitro* and *in vivo* indicates that OTA can damage plant DNA indirectly.

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REFERENCES

Bendele A.M., Neal S.B., Oberly T.J., Thompson C.Z., Bewsey B.J., Hill L.E., Rexroat M.A., Carlton W.W., Probst G.S. (1985) Evaluation of ochratoxin A for mutagenicity in a battery of bacterial and mammalian cell assays. *Food Chemical Toxicology*, **23**, 911–918.

Berry L., Brock M. (1946) Polar distribution of respiratory rate in the onion root tip. *Plant Physiology*, **21**, 542–549.

Bolle P., Mastrangelo S., Tucci P., Evandri M. (2004) Clastogenicity of atrazine assessed with the *Allium cepa* test. *Environmental and Molecular Mutagenesis*, **43**, 137–141.

Cordoba-Pedregosa M., Cordoba F., Villalba J., Gonzalez Reyes J. (2003) Zonal changes in ascorbate and hydrogen peroxide content, peroxidase and ascorbate-related enzyme activities in onion roots. *Plant Physiology*, **131**, 697–706.

Cristea I., Esposti M. (2004) Membrane lipids and cells death: an overview. *Chemical Physiology Lipids*, **129**, 133–160.

Dörrenhaus A., Föllmann W. (1997) Effects of ochratoxin A on DNA repair in cultures of rat hepatocytes and porcine urinary bladder epithelial cells. *Archives of Toxicology*, **71**, 709–713.

Ehrlich V., Darroudi F., Uhl M., Steinkellner H., Gann M., Majer B.J., Eisenbauer M., Knasmüller S. (2002) Genotoxic effects of ochratoxin A in human-derived hepatoma (HepG2) cells. *Food and Chemical Toxicology*, **40**, 1085–1090.

Feretti D., Zerbini A., Zani C., Ceretti E., Moretti M., Monarca S. (2007) *Allium cepa* chromosome aberration and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. *Food Additives and Contaminants*, **24**, 561–572.

Fiskesjo G. (1985) The *Allium* test as a standard in environmental monitoring. *Hereditas*, **102**, 99–112.

Fiskesjo G. (1988) The *Allium* test – an alternative in environmental studies: the relative toxicity of metal ions. *Mutation Research*, **197**, 243–260.

Fiskesjo G. (1993) The *Allium* test in wastewater monitoring. *Enviromental Toxicology and Water Quality*, **8**, 291–298.

Grant W. (1982) Chromosome aberration assays in *Allium* A report of the US Environmental Protection Agency gene tox program. *Mutation Research*, **99**, 273–291.

Grant W. (1994) The present status of higher plants bioassays for the detection of environmental mutagens. *Mutation Research*, **310**, 175–185.

JECFA, 2001. Safety evaluation of certain mycotoxins in food. *World Health Organization Food Additives Series*, Vol. **47**. World Health Organization, Geneva, Switzerland.

Kuczuk M.H., Benson P.M., Heath H., Hayes A.W. (1978) Evaluation of the mutagenic potential of mycotoxins using *Salmonella typhimurium* and *Saccharomyces cerevisiae*. *Mutation Research*, **53**, 11–20.

Lerda D. (1992) The effect of lead on *Allium cepa* L. *Mutation Research*, **281**, 89–92.

Mally A., Pepe G., Ravoori S., Fiore M., Gupta R.C., Dekant W., Mosesso P. (2005) Ochratoxin A causes DNA damage and cytogenetic effects but no DNA adducts in rats. *Chemical Research Toxicology*, **18**, 1253–1261.

Nielsen M., Rank J. (1994) Screening of toxicity and genotoxicity in wastewater by the use of *Allium* tests. *Hereditas*, **121**, 249–254.

Smaka-Kinel V., Stegnar P., Lovka M., Toman M. (1996) The evaluation of waste, surface and ground water quality using the *Allium* test procedure. *Mutation Research*, **368**, 171–179.

Wehner F.C., Thiel P.G., van Rensburg S.J., Demasius I.P. (1978) Mutagenicity to Salmonella typhimurium of some Aspergillus and Penicillium mycotoxins. *Mutation Research*, **58**, 193–203.

WHO (World Health Organization) 2008. Safety evaluation of certain food additives and contaminants. *World Health Organization Food Additives Series*, vol. **59**. International Programme on Chemical Safety, Geneva, Switzerland.

Würgler F.E., Friederich U., Schlatter J. (1991) Lack of mutagenicity of ochratoxin A and B, citrinin, patulin and cenestine in *Salmonella typhimurium* TA102. *Mutation Research*, **261**, 209–216.