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Food and Chemical Toxicology 43 (2005) 755–764
www.elsevier.com/locate/foodchemtox

Cytotoxicity of *Fusarium* mycotoxins to mammalian cell cultures as determined by the MTT bioassay

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Received 18 April 2004; accepted 26 January 2005

Abstract

Fusarium mycotoxins occur worldwide in cereal grains and animal feeds and cause outbreaks of *Fusarium* mycotoxicoses in humans and animals. In this study mammalian cell cultures were used to screen the cytotoxicity of the most common *Fusarium* mycotoxins; deoxynivalenol (DON), zearalenone (ZEN), fumonisin B_1 (FB_1) and moniliformin (MON). The most sensitive cell line for each *Fusarium* mycotoxin was determined for further toxicological investigations as an alternative to whole animal testing. Chinese hamster ovary cells (CHO-K1) were found to be the most sensitive for DON and FB_1 with IC_{50} values of 0.27 and 85.5 μg/ml, respectively, after 48-h exposure. The hepatocellular carcinoma cells (HepG2) showed the highest sensitivity to MON with IC_{50} values of 39.5 for 48 h and 26.8 μg/ml for 72-h exposure. Balb/c mice keratinocyte cell line (C5-O) was found to be the most sensitive to ZEN with IC_{50} of 24.1 μg/ml after 72-h exposure. DON was found the most cytotoxic to the cell cultures of all the mycotoxins tested, followed by MON, ZEN, and FB_1. The results indicated that CHO-K1, C5-O, and HepG2 cells were found to be the sensitive cell lines for preliminary screening of DON, ZEN and MON contaminated feed and food extracts, respectively.

Keywords: Deoxynivalenol; Zearalenone; Fumonisin B_1; Moniliformin; MTT bioassay; *Fusarium* mycotoxins

1. Introduction

Fusarium mycotoxins are secondary metabolites produced by numerous species of *Fusarium* that occur naturally worldwide in a variety of animal feeds and human foodstuffs. Spontaneous outbreaks of *Fusarium* mycotoxicoses in humans and animals have been reported in Europe, Asia, Africa, New Zealand and South America (Placinta et al., 1999). The most important *Fusarium* toxins that may potentially affect human and animal health and productivity are DON, ZEN, FB_1 and MON (D'Mello et al., 1999).

DON or vomitoxin, is a type B trichothecene, associated primarily with *Fusarium graminearum* (perfect stage, *Gibberella zeae*) and *Fusarium culmorum*, both of which are important plant pathogens which cause *Fusarium* head blight in wheat and *Gibberella* ear rot in maize (McLean, 1996). DON reduces growth and feed consumption (anorexia) at low concentrations in the diet whereas it induces vomiting (emesis) at higher acute doses (Rotter et al., 1996). The ingestion of DON in mice mimics the common human glomerulonephritis, IgA nephropathy (Dong and Pestka, 1993). DON is a potent protein synthesis inhibitor that can

Abbreviations: C5-O = Balb/c mice keratinocyte cell line; Caco-2 = human adenocarcinoma colon cell line; CHO-K1 = Chinese hamster ovary fibroblast cell line; DON = deoxynivalenol; FB_1 = fumonisin B_1; HepG-2 = hepatocellular carcinoma cell line; MON = moniliformin; V79 = Chinese hamster lung fibroblast cell line; ZEN = zearalenone.

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[1] A contribution of the University of Nebraska Agricultural Research Division, Lincoln, NE 68583. Journal Series No. 14536.

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doi:10.1016/j.fct.2005.01.016

significantly alter humoral immunity, cell-mediated immunity, and host resistance in a variety of experimental animal models (Pestka and Bondy, 1990). ZEN is a non-steroidal estrogenic mycotoxin, mainly produced by *F. graminearium* and *F. culmorum* found in a variety of host plants and soil debris around the world (Tanaka et al., 1988). ZEN is usually non-lethal to animals, but it is important to livestock producers because its hyperestrogenic effects adversely influence the reproductive performance of animals. ZEN has high binding affinity for the intra-cellular estrogen receptor (ER) and can enhance the proliferation of estrogen responsive tumor cells (Martin et al., 1978). There have been suggestions of the involvement of ZEN in human cervical cancer and premature initial breast development (Hsieh, 1989). Fumonisins are a group of naturally occurring mycotoxins, the most common being the B series (fumonisins B_1, B_2, and B_3), mainly produced by *Fusarium verticillioides*, *Fusarium proliferatum*, and other related species (Nelson et al., 1992) which are well-known pathogens of corn, causing stalk and ear rot worldwide (Leslie et al., 1990). Fumonisins have been shown to cause fatal animal diseases such as equine leucoencephalomalacia in horses (Kellerman et al., 1990), porcine pulmonary edema in swine (Harrison et al., 1990), and liver cancer in rats (Gelderblom and Snyman, 1991). Fumonisins have been associated with the high incidence of human esophageal cancer in regions of South Africa (Marasas et al., 1988), China (Chu and Li, 1994), and the Southeastern United States (Rheeder et al., 1992). The mode of action of fumonisins is primarily explained by interference with the *de novo* synthesis of complex glyco-sphingolipids by inhibition of ceramide synthase (Wang et al., 1991). This inhibition leads lipid-mediated alterations in signalling and metabolic pathways crucial to cell growth, apoptosis, differentiation, morphology, and endothelial cell permeability observed both in vivo and in vitro models (Riley et al., 1996). Moniliformin is produced mainly by *F. proliferatum* and *Fusarium subglutinans* and a number of *Fusarium* species. Moniliformin is a potent cardiotoxic mycotoxin in broiler chickens (Ledoux et al., 1995), and hepatotoxic in turkeys (Broomhead et al., 2002). The molecular mechanism of MON toxicity is inhibition of mitochondrial pyruvate and α-ketoglutarate oxidations, thus preventing entrance of pyruvate and α-ketoglutarate into the tricarboxylic acid cycle with subsequent reduction of oxidative phosphorylation (Thiel, 1978).

Numerous bioassays have been developed for preliminary screening of *Fusarium* mycotoxins in contaminated cereal extracts. In vitro MTT cell culture assay is one of the most used for preliminary screening since Mosmann (1983) developed. It determines the ability of viable cells to convert a soluble yellow tetrazolium salt (MTT) into insoluble purple formazan crystals by the mitochondrial dehydrogenase enzymes. The MTT bioassay is a rapid, versatile, quantitative, and highly reproducible colorimetric assay for mammalian cell viability/metabolic activity; it is useful on a large scale mycotoxins screening assay (Ciapetti et al., 1993; Hanelt et al., 1994; Dombrink-Kurtzman et al., 1994). Although a variety of mammalian cell cultures such as baby hamster kidney (BHK-21), Mardin Darby canine kidney (MDCK) (Vesonder et al., 1993), and swine kidney (SK) (Hanelt et al., 1994; Langseth et al., 1997) cell lines were used to detect *Fusarium* mycotoxins in contaminated extracts, none of them showed high sensitivity to all of the *Fusarium* mycotoxins tested. There is still a need to find a sensitive cell culture model which can highly respond to all *Fusarium* mycotoxins for the preliminary screening of *Fusarium* species contaminated extracts; therefore, it may allow to evaluate possible toxicological interactions due to the natural co-occurrence of *Fusarium* mycotoxins. The purpose of this study was to screen the cytotoxicity of the most commonly found *Fusarium* mycotoxins, DON, ZEN, FB_1, and MON, using the CHO-K1, a human adenocarcinoma colon (Caco-2), C5-O, Chinese hamster lung fibroblast (V79), and HepG2 cell lines to find the most sensitive cell line for each *Fusarium* mycotoxin as an in vitro model for further toxicological investigations as possible alternatives to whole animal testing.

2. Materials and methods

2.1. Preparation of Fusarium mycotoxins

DON, ZEN, FB_1, and MON standards were purchased from Sigma Chemical Co. (St. Louis, MO). Standards were dissolved in methanol and stored at −20 °C until they used. On the day of exposure, the standards from the stock solutions were transferred to amber vials and evaporated under streams of nitrogen then resuspended in media containing 0.1% ethanol. The standards were filtered through 0.22 μm syringe filter (Acrodisc, HT Tuffryn, Gelman Sciences, Ann Arbor, MI) to sterilize and diluted serially.

2.2. Cell lines and culture conditions

CHO-K1, Caco-2, C5-O, V79, and HepG2 cell lines were obtained from Dr. T. Lawson (Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE). The CHO-K1, Caco-2, C5-O, and V79 cell lines were used at passage numbers between 30 and 50, and HepG2 cell line was used at passage numbers between 80 and 100. The Caco-2, C5-O, V79 cells in Dulbecco's Modified Eagle Medium (DMEM), CHO-K1 cells in DMEM/F-12 and HepG2 cells in Minimum Essential Medium

(MEM) were grown as monolayers in 80 cm^2 culture flasks. Media were supplemented with 1.5 g/l sodium bicarbonate, 0.11 g/l sodium pyruvate, 1% non-essential amino acid (NEAA), 25 mM HEPES, antibiotic-antimycotic; 100 units of penicillin/ml, 100 μg of streptomycin/ml, and 25 ng amphotericin B/ml, and 10% fetal bovine serum (FBS). Cell culture media and their supplements were purchased from Life Technologies Gibco BRL Products (Rockville, MD). The cell cultures were maintained in a humidified atmosphere of 5% CO_2 at 37 °C. The cell lines were harvested when they reached 80% confluence to maintain exponential growth.

2.3. The MTT cytotoxicity assay of Fusarium mycotoxins

The CHO-K1, Caco-2, C5-O, V79, and HepG2 mammalian cell lines were used to evaluate dose–response relationships of DON, ZEN, FB_1, and MON. The cell monolayers in exponential growth were harvested using 0.25% trypsin (10 mM, trypsin-EDTA 0.25% trypsin, 1 mM EDTA.4Na) and single-cell suspensions were obtained by repeated pipetting. The cells were counted with a hemacytometer (Hausser Scientific, Horsham, PA) and single cell suspensions containing cell densities ranging from 1×10^2 to 5×10^4 cells per 200 μl medium/well were added to 96-well plates by serial dilution then the MTT bioassay was performed concomitantly. The number of seeded cells was determined from the linear correlation between the number of seeded cells and the optical density (OD) values. Caco-2, C5-O, and HepG2 at a cell density of 1×10^4 cells, CHO-K1 and V79 at 5×10^3 cells per 100 μl medium were seeded to each well of the 96 wells plates (Nunclon™, VWR International Inc., MD) and incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. Mycotoxin standards in 100 μl of medium were added from high to low concentrations to the wells. Final concentrations of standards in the wells were adjusted and ranged from 0.04 to 20 μg/ml DON, and 0.2 to 100 μg/ml ZEN, FB_1, and MON. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was purchased from Sigma Chemical Co. (St. Louis, MO). It was dissolved in phosphate buffer saline (PBS) solution at concentration of 5 mg/ml and filtered through a 0.22 μm filter to sterilize and remove insoluble residues then stored in the amber vials at 4 °C for a month. After 48 and 72 h incubation, 25 μl of the MTT solution was added to each well of 96-well plates and incubated for 4 h at 37 °C in a humidified atmosphere of 5% CO_2. At the end of the incubation period, the media were discarded using a suction pump. The extraction buffer of 20% w/v sodium dodecyl sulfate (SDS) (Sigma Chemical Co., St. Louis, MO) in a solution of 50% of *N*,*N*-dimethylformamide (DMF) (Sigma Chemical Co., St. Louis, MO) in demineralized water (50:50, v/v) was prepared at pH 4.7 and filtered through a 0.22 μm filter to remove insoluble residues. The extraction buffer in 100 μl of 20% w/v of SDS (Hansen et al., 1989) was added into each well of the 96-well plates to solubilize formazan crystals. The culture plates were placed on an orbital shaker at 37 °C for overnight. The absorbance was measured at the test wavelength of 570 nm and the reference wavelength of 690 nm by a microplate reader (Tecan Sunrise Absorbance Microplate Reader, Pheonix, CA). The positive control contained an adjusted seeding cell number in log phase in the culture medium containing 0.1% ethanol. Assays with each cell line and mycotoxin, and each exposure time were repeated three times.

2.4. Calculations

The number of seeded cells was determined from the linear correlation between the number of seeded cells and the OD values. The % inhibition of cell proliferation were calculated using the formula: $\%IC = [1\text{-}(A_{570test}/A_{570cont})] \times 100$ where % IC = % inhibition of cell proliferation, $A_{570test}$ = absorbance of test sample, $A_{570cont}$ = absorbance of control sample (Dombrink-Kurtzman et al., 1994). Dose-response curves were plotted from %IC values versus concentrations of mycotoxins, μg/ml, (*x*-axis, log scale). The values of 50% inhibition of cell proliferation (IC_{50}) were calculated by locating the *x*-axis values corresponding to one-half the absorbance values of positive control containing an adjusted seeding cell number and 0.1% ethanol without addition of any mycotoxin.

3. Results

The optimal cell number seeding for each cell line (since cells during the assay have to be actively metabolizing), and the duration of the experiment for each mycotoxin were determined. Linear regression analysis revealed high correlation between the OD signals and the seeded cell numbers ($r = 0.914 - 0.988$) following 72 h incubation (Fig. 1). The numbers of seeding cells for the MTT bioassay were determined for Caco-2, C5-O, and HepG2 at a cell density of 1×10^4 cells, for CHO-K1 and V79 at 5×10^3 cells per well. To allow sufficient time for mycotoxins induced cell death, the duration of the experiment upon dose–response relationships of the cell lines was determined 48 h for DON, and 48 or 72 h for ZEN, MON, and FB_1 due to their low acute toxicity. The DON exposure time was also performed as 24 h using only CHO-K1 cell line to evaluate its high acute toxicity. The cytotoxic effects of *Fusarium* mycotoxins, DON, ZEN, FB_1 and MON causing 50% inhibition of cell proliferation to five established cell lines were determined by the MTT bioassay (Table 1). The dose–response relationships for the cytotoxic effects of DON, ZEN, FB_1 and MON to CHO-K1, Caco-2,

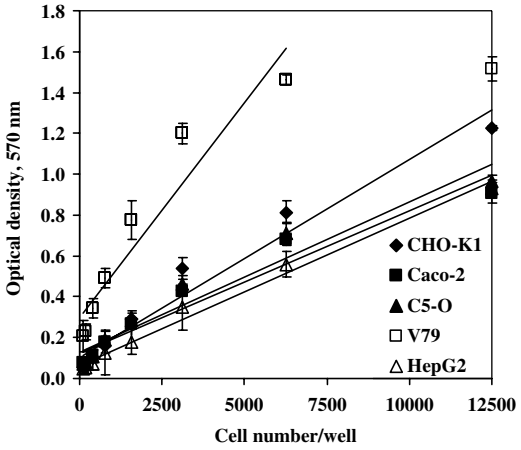

Fig. 1. Correlation of optical density and cell number in CHO-K1, Caco-2, C5-O, V79, and HepG2 cell lines after 72 h incubation as measured by the MTT bioassay. The correlation coefficients (r) for the linear regression equations were calculated for each cell line (CHO-K1, $r = 0.958$; Caco-2, $r = 0.941$; C5-O, $r = 0.939$; V79, $r = 0.914$; HepG2, $r = 0.988$). Each point represents the mean ± SD of three replicates.

C5-O, V79, and HepG2 mammalian cell lines were evaluated following 48-h exposures (Fig. 2). DON was found to be the most cytotoxic among the tested *Fusarium* mycotoxins due to potent inhibition of protein synthesis with IC_{50} values ranging from 0.27 to 8.36 μg/ml to all mammalian cell lines tested. The CHO-K1 cell line showed the highest response to DON with IC_{50} value of 1.15 and 0.27 μg/ml following 24- and 48-h exposure, respectively. The IC_{50} value of DON for 48 h was found to be 4-fold higher than the 24-h exposure period. Sensitivities of cell lines to DON were found in decreasing order of CHO-K1 > V79 > C5-O > Caco-2 > HepG2 cells with IC_{50} values of 0.27, 0.49, 0.54, 1.02, and 8.36 μg/ml, respectively, after 48-h exposure (Fig. 2-A). The DON cytotoxicity followed in order by MON in HepG2 cells > ZEN in C5-O cells > FB_1 in CHO-K1 cells with IC_{50} values of 39.5, 85.5, >100 g/ml, respectively, after 48 h duration time. The cell lines were also exposed to ZEN, FB_1, and MON for 72 h to evaluate the fate of dose–response relationships in longer duration time due to their low cytotoxicity as compared to the DON cytotoxicity (Fig. 3).

ZEN was found to be less cytotoxic than the other tested *Fusarium* mycotoxins among the screened cell lines after 48-h exposure however; ZEN at maximum concentration of 100 μg/ml caused inhibition of HepG2, C5-O, Caco-2, V79, and CHO-K1 cell proliferation by 46%, 41% 33%, 27%, and 24%, respectively (Fig. 2-B). After 72 h incubation, C5-O cell line showed the highest response to ZEN cytotoxicity followed by Caco-2 cells with IC_{50} of 24.1 and 43.7 μg/ml, respectively, whereas the IC_{50} value of ZEN on the other target HepG2, V79, and CHO-K1 cell lines were found to be higher than 100 μg/ml (Fig. 3A). The sensitivities of the cell lines were found to be in the decreasing order of C5-O > Caco-2 > HepG2 > V79 > CHO-K1 cells.

FB_1 showed different trend in its dose–response relationship on tested mammalian cell lines at different duration times. FB_1 caused higher response on mammalian cell lines tested at concentrations of >50 μg/ml for 48 h than 72-h exposure period. After 48-h exposure, the CHO-K1 cell line was found to be the most sensitive to FB_1 cytotoxicity at high concentrations (IC_{50} of 85.8 μg/ml), whereas the V79 cell line showed higher sensitivity to FB_1 at lower concentrations of <25 μg/ml (IC_{50} of 98.2 g/ml) (Fig. 2C). The Caco-2 and C5-O cell lines showed similar low response to FB_1 cytotoxicity. After 72-h exposure, FB_1 at concentration of 100 μg/ml caused inhibition of HepG2 and V79 cell proliferation by 39% and 38%, respectively, followed in decreasing order by CHO-K1 (30%) > C5-O (27%) > Caco-2 (19%) cell lines (Fig. 3B).

MON was found moderately cytotoxic to the tested mammalian cell cultures. The HepG2 cell line was found to be the most sensitive cell line tested to examine the MON cytotoxicity with IC_{50} values of 39.5 and 26.8 g/ml after 48- and 72-h exposure, respectively. The cytotoxic effect of MON at the highest concentration of 100 μg/ml to Caco-2 and C5-O cells resulted in less than 30% inhibition of cell proliferation whereas the V79 and CHO-K1 cells were inhibited by 10% after 48-h expo-

Table 1
The IC_{50} values of DON, ZEN, FB_1, and MON following 48- and 72-h exposure measured by the MTT bioassay using CHO-K1, Caco-2, C5-O, V79 and HepG2 cell lines

IC_{50} values in μg/ml and μM given in parentheses						
Mycotoxins	Time (h)	CHO-K1	Caco-2	C5-O	V79	HepG2
DON	48	0.27[a] (0.91)	1.02 (3.40)	0.54 (1.82)	0.49 (1.55)	8.36 (28.2)
ZEN	48	>100 (313)	>100 (313)	>100 (313)	>100 (313)	>100 (313)
	72	>100 (313)	43.7 (137)	24.1 (75.5)	>100 (313)	>100 (313)
FB_1	48	85.5 (118)	94.8 (131)	>100 (138)	98.3 (136)	>100 (138)
	72	>100 (138)	>100 (138)	>100 (138)	>100 (138)	>100 (138)
MON	48	>100(1020)	>100 (1020)	>100 (1020)	>100 (1020)	39.5 (403)
	72	>100 (1020)	30.9 (315)	34.2 (349)	>100 (1020)	26.8 (273)

[a] %IC_{50} values calculated by locating the x-axis values which are the DON, ZEN, FB_1, or MON concentrations, μg/ml, corresponding to one-half the OD values of controls. Standard deviations for all data points were <5% the value of the point.

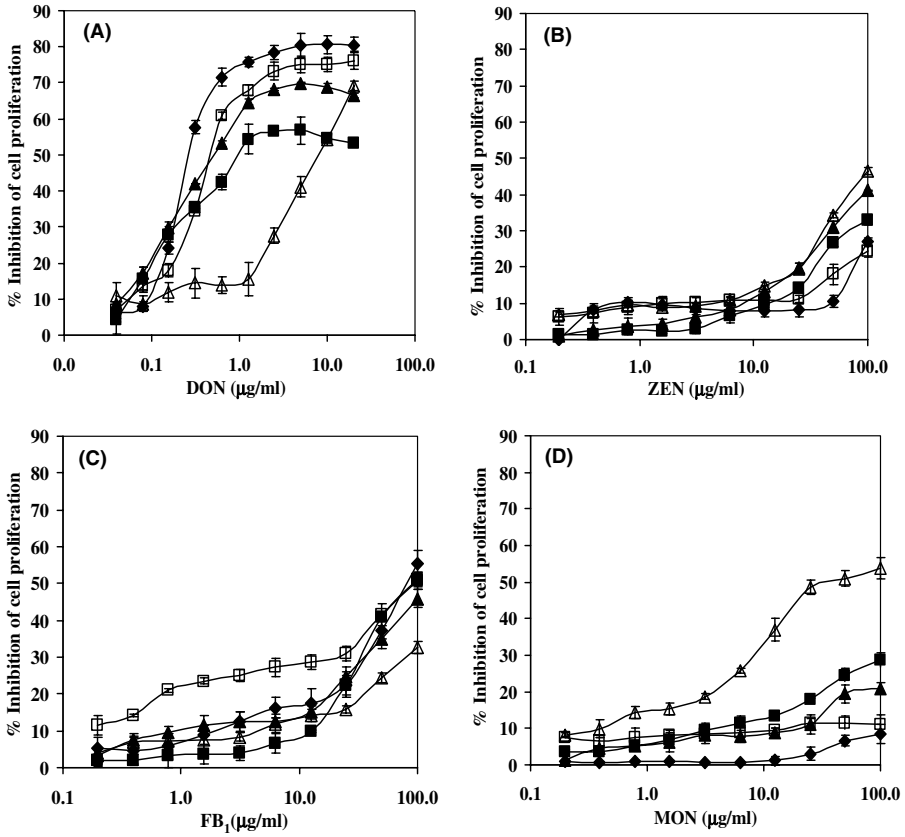

Fig. 2. The cytotoxic effects of DON (A) at concentrations of 0.04–20 μg/ml, and ZEN (B), FB_1 (C), and MON (D) at concentrations of 0.2–100 μg/ml on proliferation of CHO-K1 (◆), Caco-2 (■), C5-O (▲), V79 (□), and HepG2 (△) cell lines following 48-h exposure as determined by the MTT bioassay. Standard deviations for all data points were <5% the value of the point. Each point represents the mean ± SD of three replicates.

sure (Fig. 2D). The HepG2, Caco-2 and C5-O cell lines showed very similar high response to the MON cytotoxicity, but the CHO-K1 and V79 cells showed less sensitivity after 72-h exposure (Fig. 3C). The sensitivities of the cell lines were found in the decreasing order of HepG2 (IC_{50} 26.8 μg/ml) > Caco-2 (IC_{50} 30.9 μg/ml) > C5-O (IC_{50} 34.2 μg/ml) > CHO-K1 (IC_{50} > 100 μg/ml) > V79 (IC_{50} > 100 μg/ml) cell lines.

4. Discussion

Over the last few decades several in vitro assays using mammalian cell cultures have been developed thus avoiding the excessive use of laboratory animals which is expensive, time consuming, and often involves ethical problems. Cell culture systems can be more sensitive and more reproducible than tests involving intact animals (Buckle and Senders, 1990). These cell culture assays can be used for the preliminary screening of toxicity of mycotoxins as well as evaluating structure–activity relationships and elucidating the modes of action of toxins at the biochemical level related to cellular organelles and are used as toxicity markers (Rodrâiguez and Haun, 1999). In cell lines, in vivo enzymes of detoxification or activation, required many organic substances, were missing therefore, specific assays exploring specific intra-cellular mechanisms could lead to overestimating the risks. The comparison of toxic responses obtained with each bioassay may orient to its toxicological mechanism (Sauvant et al., 1995). Comparison of in vitro and in vivo biological activity of mycotoxins demonstrated significant relationship between the two tests (Terse et al., 1993; Sauvant et al., 1995; Abbas et al., 1984). The cytotoxic effect of fungal extracts on Swiss mouse 3T3 fibroblasts and human diploid skin GM3349 fibroblast cell lines correlated well with weight loss and feed refusal observed in rat feeding studies (Abbas et al., 1984). Cytotoxicity tests generally possess a broad spectrum of sensitivity and are able to detect many mycotoxins which are potentially inhibit the biochemical activity of a variety of cell cultures of animal and human origin. Cytotoxicological characterizations of *Fusarium* species contaminated cereal extracts were screened to compare their toxin production by the MTT bioassay using mammalian cell cultures (Langseth et al., 1999; Langseth et al., 1997; Abeywickrama and Bean, 1992). It was showed that the MTT bioassay is an alternative to assay that measure DNA replication based on ^{3}H-thymidine incorporation, and thus, avoids the necessity of

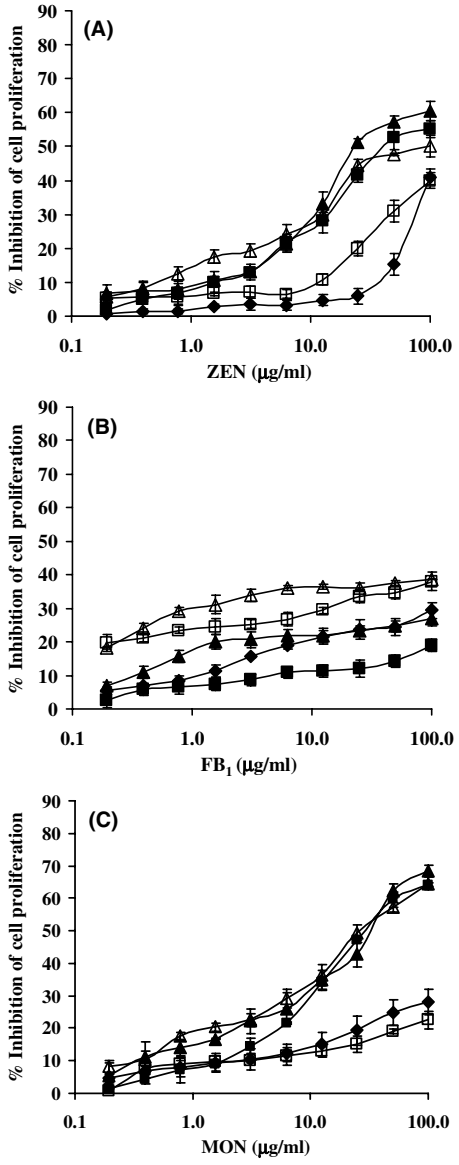

Fig. 3. The cytotoxic effects of ZEN (A), FB_1 (B), and MON (C) at concentrations of 0.2–100 μg/ml on proliferation of CHO-K1 (◆), Caco-2 (■), C5-O (▲), V79 (□), and HepG2 (△) cell lines following 72-h exposure as determined by the MTT bioassay. Standard deviations for all data points were <5% the value of the point. Each point represents the mean ± SD of three replicates.

handling radioactive materials (Ciapetti et al., 1993; Denizot and Lang, 1986; Dombrink-Kurtzman et al., 1994). Charoenpornsook et al. (1998) reported that the MTT bioassay was found to be less sensitive than lactate dehydrogenase (LDH) assay to examine *Fusarium* mycotoxin cytotoxicity. On the other hand the MTT bioassay showed better correlation with in vivo assay than the LDH assay (Faller et al., 2002). Measurement of cell growth by MTT reduction correlated well with indices of cellular protein and viable cell number. It provides an accurate measure of the total cell number rather than being limited to the detection of subpopulations of actively dividing cells (Loveland et al., 1992). The MTT bioassay has been extensively used to evaluate the cytotoxicity of DON, ZEN (Rotter et al., 1993; Hanelt et al., 1994; Visconti et al., 1991), FB_1 (Dombrink-Kurtzman et al., 1994; Shier et al., 1991) and MON (Wu et al., 1995). The results of this study also indicated that the MTT bioassay using mammalian cell lines was useful for qualitative and quantitative analyses of the *Fusarium* mycotoxins, DON, ZEN, FB_1 and MON.

The cytotoxicological characterization of *Fusarium* mycotoxins tested showed a cytotoxicity range from high to relatively low when compared to each other. DON was found to be highly cytotoxic whereas MON and ZEN were moderately and FB_1 was found to be less cytotoxic within the tested concentration range and the time periods as compared to the other mycotoxins tested. In this study DON showed high acute toxicity therefore, the exposure period was chosen as 48 h but ZEN, FB_1, and MON were additionally tested for 72 h due to their low acute toxicity to evaluate their fate of toxicity in a longer exposure period. In previous studies, in vitro cell cultures also showed different susceptibility to DON cytotoxicity measured at different experimental periods by different cytotoxicity assays. DON inhibits protein synthesis in African Green Monkey kidney (Vero), and murine erythroleukemia cells (Ehrlich and Daigle, 1987) at the ribosomal level, due to the presence of an intact 9,10-double bond and the C-12, 13 epoxide by inhibition of chain elongation. It was showed that the metabolism of DON to de-epoxy metabolite significantly decreased the cytotoxicity of DON to pig kidney cells (Kollarczik et al., 1994). In this study the HepG2 cell line was found to be the least sensitive to DON cytotoxicity within the tested cell lines. It might be due to the conversion of DON to less toxic de-epoxide metabolite. Although DON is one of the least cytotoxic trichothecene comparing to others, it has been found cytotoxic to the number of cell cultures. The comparison among the cell lines SK, Helene-Langer (HeLa), and MDCK cells showed different susceptibility to DON measured, with 80% of MTT cleavage activity at levels of 0.8, 100, and >200 μg/ml, respectively, for 24-h exposure (Hanelt et al., 1994). Reubel et al. (1989) reported that DON at concentrations of 10 and 100 μg/ml showed suppressive effect on the activity of SK, MDCK, VERO, and bovine embryonic lung (BEL) cell lines, depending on the incubation time. Rotter et al. (1993) reported an IC_{50} value of DON on BHK-21 cells measured by the MTT bioassay was found at concentration of 0.112 μg/ml after 24-h exposure. The cytotoxic effects of DON measured by the MTT bioassay using another swine kidney (PK15) cells (Yike et al., 1999) and *Xeroderma pigmentosum* (XP R012) human fibroblasts cells (Robbana-Barnat et al., 1988) were found to inhibit 50% of cells at concentrations of 1.47 μg/ml for 72 h and 0.252 μg/ml after 48-h exposure, respectively. The

human erythroleukemia (K-562) and lymphoid B (MIN-GL1) cell lines were also found to be sensitive to DON cytotoxicity with IC_{50} values of 0.3 and 0.4 μg/ml, respectively (Visconti et al., 1991). Yang et al. (2000) showed close correlation between the cytotoxic and apoptotic capacities of DON using murine macrophage RAW 264.7 and human leukemic U937 cell lines. The results of previous studies showed that BHK-21 cell line was the most sensitive in vitro model to evaluate the DON cytotoxicity, and followed by XP RO12 > K-562 > MIN-GL1 > SK > PK15 cell lines. In this study, the CHO-K1 was found to be the best cell line to examine the DON cytotoxicity and followed by V79 > C5-O > Caco-2 > HepG2 cells.

ZEN stimulated the proliferation of estrogen responsive tumor cells at low concentrations whereas it may a potent inhibitor of protein tyrosine kinases and can inhibit cell cycle progression in tumor cells at high concentrations (Withanage et al., 2001). The results of ZEN cytotoxicity showed that it was less cytotoxic to the cell cultures tested after 48 h, while it caused increasing cytotoxic effect on C5-O and Caco-2 cell lines after 72-h exposure. The V79 and CHO-K1 cell lines were found to be less sensitive to ZEN during the both exposure periods. The increased dose–response relationships of ZEN on the cell cultures were observed at concentrations between 25 and 100 μg/ml. In previous studies, ZEN was also found not cytotoxic to the other cell culture models such as SK, MDCK, and HeLa cells up to the highest concentrations of 50, 100, and 100 μg/ml, respectively, after 24 h incubation (Hanelt et al., 1994). However, cytotoxic effects of 100 μg ZEN/ml were reported in 80% inhibition of MDCK and SK cells while ZEN at low concentrations between 0.1 and 10 μg/ml resulted in stimulation of both cell lines. ZEN at concentration of 100 μg/ml caused 40% inhibition on BEL and VERO cell lines after 16-h exposure (Reubel et al., 1989). BHK-21 and a human epidermoid carcinoma (HEP-2) cells also were not found susceptible to ZEN at low concentrations (Abeywickrama and Bean, 1992; Robb et al., 1990). ZEN caused 50% inhibition in bovine (BE 12-6) and murine (NIH/3T3) embryonic cell lines at concentrations of 2.4 and 23.72 μg/ml, respectively, after 72-h exposure (Terse et al., 1993). The cytotoxicity of ZEN on K-562, and MIN-GL1 cells was found with IC_{50} values of 10 and > 10 μg/ml, respectively, after 48-h exposure (Visconti et al., 1991). The most sensitive in vitro cell culture model to evaluate the cytotoxic effect of ZEN among the examined cell lines was found to be BE 12-6 cells followed by in order of K-562 > NIH/3T3 > SK > MDCK > BEL > VERO cell lines. As a result of this study the C5-O was found to be the most sensitive cell line to evaluate the ZEN cytotoxicity followed by Caco-2 and HepG2.

The increase in the sphinganine to sphingosine ratio was used as a biomarker for FB_1 exposure which disrupted the biosynthesis of sphingolipids by inhibition of ceramide synthase. The free sphinganine levels in primary rat hepatocytes and in renal epithelial cells (LLC-PK1) were significantly increased after exposure to FB_1 (Yoo et al., 1992; Norred et al., 1992). The accumulation of sphinganine levels in the cell cultures after exposure to FB_1 at low concentrations were found to be non-cytotoxic due to elimination, suggesting rapid metabolism but prolonged exposure to FB_1 at high concentrations caused increased cell death (Riley et al., 1996). The inhibition of sphingolipid biosynthesis by FB_1 was growth-inhibitory and cytotoxic for the cells secondary to apoptotic cell death in a time and dose dependent manner (Riley et al., 1996). Norred et al. (1997) reported that the disruption of sphingolipid metabolism was a specific cytotoxic response. In this study, the cytotoxic effects of FB_1 could be used as an alternative biomarker to screen the FB_1 toxicity; however, FB_1 showed a wide variety of cytotoxic effects on tested mammalian cell cultures. FB_1 at low concentrations was found less cytotoxic to all tested mammalian cell lines; however, it caused 50% inhibition of proliferation of CHO-K1, Caco-2 and V79 cell lines at high concentrations of 85.5, 94.8, and 98.3 μg/ml, respectively, after exposure to FB_1 for 48 h. The CHO-K1 cell line was found to be the most sensitive to FB_1 cytotoxicity at high concentrations whereas the V79 cell line showed higher sensitivity to FB_1 at lower concentrations of <25 μg/ml (Fig. 2-C). The Caco-2 and C5-O cell lines showed similar trend to FB_1 cytotoxicity. After 72-h exposure, FB_1 at maximum concentration of 100 μg/ml caused inhibition of HepG2 and V79 cell proliferation by 39% and 38%, respectively, followed in decreasing order by CHO-K1 (30%) > C5-O (27%) > Caco-2 (19%) cell lines. The FB_1 cytotoxicity was found to be higher at 48 h than 72-h exposure period. In vivo study showed that the elevation of sphinganine was reversible after the short term FB_1 exposure (Wang et al., 1992). The result of this assay might explain the decreasing trend of the cytotoxicity of FB_1 after 72-h exposure. Yoo et al. (1992) reported that the increase in sphinganine concentration reached maximum level at 48-h exposure to FB_1 because there was a lag period of approximately 48 h proceeding inhibition of cell proliferation. FB_1 was also found to be not cytotoxic to SK cells (Hanelt et al., 1994), BHK-21, CHO-K1, MDCK, rat hepatoma (RH), and McCoy mause (MM) fibroblast cell lines at concentration of 100 μg/ml (Vesonder et al., 1993). Conversely, FB_1 was found to be inhibited cell proliferation in different cell lines, including rat hepatoma (H4TG), MDCK, NIH3T3, and LLC-PK1 cell lines (Abbas et al., 1995; Yoo et al., 1992). Shier et al. (1991) reported that among the 15 mammalian cell lines, MDCK and H4TG cell lines were found to be more sensitive to cytotoxic effects of FB_1 at levels of IC_{50} values 2.5 and 4 μg/ml, respectively, after 4 days exposure. The MDCK cell line might

be the best cell culture model for FB_1 cytotoxicity in spite of controversial results and followed by the H4TG cell line. As a result of this study none of the cell line was found to be highly sensitive to the FB_1 cytotoxicity at low concentrations however, the CHO-K1 showed the highest sensitivity among the cell lines tested.

The HepG2 cell line was found to be very sensitive to the cytotoxic effect of MON, followed by Caco-2 and C5-O after 72-h exposure whereas CHO-K1 and V79 cells showed low responses to the MON toxicity. The hepatoma, HepG2, and the intestine, Caco-2, cell line sensitivities were correlated with previous in vivo studies that showed hepatotoxic effects of MON in old turkeys liver (Broomhead et al., 2002) and hemorrhaging effects in the rat intestine (Abbas et al., 1990). The suggested mechanism of MON might be inhibition of mitochondrial pyruvate and α-ketoglutarate oxidations, thus preventing entrance of pyruvate and α-ketoglutarate into the tricarboxylic acid cycle. It might lead the cellular energy depletion as a mechanism of cell death caused by MON (Wu and Vesonder, 1997). In other in vitro studies, MON was also found cytotoxic to RH, MDCK, MM fibroblast cells at IC_{50} of 10, 10, and 25 μg/ml for 24–96 h exposure, respectively. Conversely, it was found to be not cytotoxic on BHK-21 cell line at levels up to 75 μg/ml and only caused 25% death of CHO-K1 cells at concentration of 5 μg/ml (Vesonder et al., 1993). MON was also found not cytotoxic to Porcine kidney epithelial (PK15) (Morrison et al., 2002) and SK cell lines (Hanelt et al., 1994). Comparison of cytotoxicity indicated that MON was more cytotoxic than FB_1 on cultured chicken primary cells (Wu et al., 1995), and on cultured mammalian cell lines (Vesonder et al., 1993). Cytotoxicity of MON in chicken primary cell cultures, skeletal myocytes, cardiac myocytes and in splenocytes were found with IC_{50} values of 42 μM, 95 μM and >200 μM for 48-h exposure but it was not cytotoxic to chondrophages and macrophages (Wu et al., 1995). Reams et al. (1996) reported that increased concentrations of MON caused increased severe cytoplasmic vacuolation and formation of cytoplasmic blebs in rat skeletal muscle myoblast (L6 myoblasts). It might be due to part of the oxidative damage and altered pyruvate metabolism (Reams et al., 1996). The reduction of the activities of glutathione peroxidase and glutathione reductase in rat myocardium due to exposure to MON resulted in increased levels of active oxygen and free radicals in the myocardium with destruction of the cell membrane, leading to cell death (Chen et al., 1990). In this study, the human hepatoma HepG2 cells was found to be the best in vitro cell culture model to evaluate the MON cytotoxicity whereas the rat hepatoma RH cells was found the most sensitive in the previous studies.

The evaluation of in vitro common cell culture models for *Fusarium* toxins is necessary to screen the naturally contaminated grain extracts and examine the interactions of toxins such as synergistic, antagonistic or additional effects to one another in the mixture even if they have different target sites in biological systems. Several *Fusarium* mycotoxins are often found in combination in infested cereal grains. There is increasing concern about the hazard of exposure to mixtures because of their natural co-occurrence. None of the cell lines tested showed a high response to all of the *Fusarium* mycotoxins, DON, ZEN, FB_1, and MON. Despite conflicting results, the BHK-21, and MDCK cell lines have potential to screen the mixture of *Fusarium* toxins. There is still a need to find the best in vitro cell culture model for *Fusarium* mycotoxins at a certain exposure time and the measurement. Different cytotoxic effects of the mycotoxins might arise from the ability of the particular mycotoxin to bind to cellular receptors and/or penetrate cell membranes, which is dependent on the size, structural conformation of the toxin molecules and the polarity of the compounds. Varying cytotoxic responses of different metabolic activity and different enzyme potential of the target cells may also be the reason of different sensitivity of cell lines. The MTT bioassay is a useful method for qualification and quantification of large numbers of *Fusarium* culture extract as well as a simplified type of cytotoxicity scoring system. It has usually higher sensitivity and lower detection limit to analyze the *Fusarium* mycotoxins than most of the chemical methods (Buckle and Senders, 1990). The main application lies in being able to screen products for biological activity and identity those samples for which a specific chemical analysis is justified. It may alert to the presence of a mycotoxin even when its chemical identity is unknown after *Fusarium* contamination or detoxification processes of feeds and foodstuffs.

Acknowledgement

This research was conducted under Project Number 16-056 of the Agricultural Research Division, University of Nebraska-Lincoln, Lincoln, Nebraska 68583, and is based upon work supported in part by grants from the Midwest Advanced Food Manufacturing Alliance (MAFMA) and the Anderson Fund of the NC 213, Multistate Research Project.

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