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PROTECTIVE EFFECT OF FOSFOMYCIN ON DEOXYNIVALENOL- TREATED CELL CULTURES

Denisa Soledad Pérez Gaudio[1,2]*; Guadalupe Martínez[1,3], María Belén Fernández Paggi[1,3], María Belén Riccio[1], Julieta María Decundo[1,3], Susana Dieguez[1], María Ofelia Tapia[1,3], Alejandro Luis Soraci[1,3]

[1]Área de Toxicología, Dpto. de Fisiopatología, CIVETAN, Facultad de Cs. Veterinarias, UNCPBA, Tandil, Buenos Aires, Argentina.
[2]Fundación Bunge y Born, Ciudad Autónoma de Buenos Aires, Argentina.
[3]CONICET - Consejo Nacional de Investigaciones Científicas y Técnicas, Ciudad Autónoma de Buenos Aires, Argentina.

*** Corresponding Author: Dr. Denisa Soledad Pérez Gaudio**
Área de Toxicología, Dpto. de Fisiopatología, CIVETAN, Facultad de Cs. Veterinarias, UNCPBA, Tandil, Buenos Aires, Argentina.

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ABSTRACT
Fosfomycin is a broad-spectrum antibiotic which inhibits cell wall synthesis as it interferes with peptidoglycan production. It has bactericidal activity against both Gram positive and Gram negative bacteria. Because its low toxicity and potential efficacy, the use of fosfomycin has been proposed for animals and humans. Fosfomycin inhibits bacterial adhesion to epithelial cells, penetrates biofilms of exopolysaccharides, promotes phagocytosis, has immunomodulatory effects, and protects against the toxicity caused by other drugs. On the other hand, deoxynivalenol causes cytotoxicity on tissues of rapid growth and fast turnover. The aim of this study was to determine the toxic dose of deoxynivalenol on HEp-2 cell cultures, to determine whether deoxynivalenol induces apoptosis in different cell cultures and to evaluate whether fosfomycin has a protective effect on the cell lines exposed to deoxynivalenol. Toxicity of deoxynivalenol on HEp-2 cells was observed with doses above 1.4 µg/mL. Cells cultures incubated with 2.8 µg/mL of deoxynivalenol and 550 µg/mL of fosfomycin were similar to untreated cells cultures, with preservation of cell monolayer and without cell damage. Apoptotic cell percentage was significantly higher for HEp-2, MDBK and BT cells treated with deoxynivalenol than for cells incubated with both the mycotoxin and the antibiotic. Differences in the percentage of apoptotic cells were not observed between BHK cell cultures treated with deoxynivalenol and for those incubated with both the mycotoxin and the antibiotic. Further studies are needed to determine whether deoxynivalenol induces apoptosis only on cells of epithelial origin and to understand the implications of fosfomycin protective effect under *in vivo* conditions.

KEYWORDS: Fosfomycin; Deoxynivalenol; Cell cultures; Apoptosis; Protective effect.

INTRODUCTION
Fosfomycin (cis-1, 2-epoxyphosphonic acid) (FOS) is a broad-spectrum antibiotic, structurally unrelated to other classes of antimicrobial agents. FOS inhibits cell wall synthesis as it interferes with peptidoglycan production at an earlier stage than beta-lactams or glycopeptide antibiotics (Popovic et al., 2009). FOS has bactericidal activity against both Gram positive and Gram negative bacteria (Gobernado, 2003; Kahan et al., 1974). When compared with other antibiotics, FOS has a broader *in vitro* spectrum of action than penicillins and semi-synthetic cephalosporins (Mata et al., 1977). Furthermore, cross-resistance has not been reported (Gobernado, 2003). The use of FOS in animals and humans has been proposed because of its low toxicity and potential efficacy (Gallego et al., 1974). It is also widely used in animal production due to its rapid effect, good tolerance and lack of side effects (Aramayona et al., 1977; Carramiñana et al., 2004). The acidic nature of FOS allows the rapid formation of salts. Calcium FOS salt is used orally, whereas the more water-soluble disodium salt can be administered intravenously. FOS-tromethamine salt is highly hydro-soluble and offers a good oral bioavailability in humans (Borsa et al., 1988; Patel et al., 1997; Popovic et al., 2009). It has been demonstrated that, besides being a bacterial inhibitor, FOS has other properties such as, inhibition of bacterial adhesion to epithelial cells, penetration of wells in the biofilms of exopolysaccharides and promotion of phagocytosis. Furthermore, it has immunomodulatory effects and protects against nephrotoxicity caused by drugs such as cisplatin, cyclosporine, aminoglycosides, vancomycin, teicoplanin, amphotericin B and polymyxin (Gobernado, 2003).

Deoxynivalenol (DON) is a mycotoxin, a toxic substance resulting from the secondary metabolism of different strains of filamentous fungi, mainly *Fusarium graminearum* and *Fusarium sporotrichiodes*. DON is a trichothecene and due to its emetic effect, it is also known as "vomitoxin" (Rotter et al., 1996). This toxin is a contaminant of wheat, corn, rice, barley, oats and other cereal grains used in food production and animal food. The main mechanisms involved in the toxic action of DON are the inhibition of protein synthesis and the cytotoxicity on tissues of rapid growth and fast turnover (Desjardins, 2006; Eriksen, 2003). Particularly, the intestinal tract and immune cells are highly sensitive to DON (Petska, 2008). It has been demonstrated that in these cell types DON induces DNA damage and apoptosis (Awad et al., 2014; Savard et al., 2015).

The aim of this study was to determine the toxic dose of DON on an epithelial cell culture routinely used for antibiotic penetration assays (HEp-2), to determine whether DON induces apoptosis in different cell cultures and to evaluate whether FOS has a protective effect on the cell lines exposed to DON.

MATERIALS AND METHODS

Cell cultures: For these experiments, HEp-2 (Human Epidermoid carcinoma strain 2; Human Epithelial type 2 cells), Madin Darby Bovine Kidney (MDBK), Bovine Turbinate (BT) and Baby Hamster Kidney (BHK) cell lines were used. HEp-2 cells constitute a useful model for studying FOS penetration (Martínez et al., 2011; Pérez et al., 2012). Cells were grown in 75 cm^2-flasks with Eagle's Minimal Essential Medium (E-MEM) and 20% of fetal bovine serum (FBS). At 24 h, the medium was replaced (MEM and 10% FBS) to allow cell division to continue up to a complete confluent monolayer. The medium was removed and cells were washed with Phosphate Buffer Saline (PBS). Trypsin was added for 10 min. at 37 º C. Detached cells were resuspended in PBS and centrifuged for 5 min. at 1500 rpm. The cell pellet was resuspended in MEM with 10% FBS and transferred to new flasks. Cells were maintained in incubator at 37 º C and 5% CO_2 and seeded in 6-well plates for each experiment at a density of 1.2 x 10^6 cells/mL, as indicated below.

Determination of FOS and DON doses for the experimental studies: For protection studies, calcium FOS salt (FosbacTM, Bedson Lab., Argentina) was used. A 600 µg/mL stock solution of calcium FOS was prepared in Milli-Q water. The different doses of FOS were prepared in saline solution.

As mycotoxin source, a pure standard of DON with a molecular weight of 296.32 g/mol (C15H20O6) (Sigma-Aldrich ®) was used. An 80 µg/mL stock solution in methanol was prepared.

To determine the toxic dose of DON on HEp-2 cells and to evaluate whether FOS has a protective effect on DON-treated cells, two different experiments were performed.

For both experiments, cells were seeded in 6 well-plates. The culture medium was removed and cells were incubated at different times, depending on the assay, at 37 º C and 5% CO_2. In the first experiment, HEp-2 cells were incubated for 24 h with increasing doses of DON (0.35, 0.7, 1.4 and 2.8 µg/mL). Six-replicates of the assay were performed. In the second experiment, cell cultures were incubated for 4 h since this was the earlier time-point at which cell detachment was observed when cells were incubated only with the mycotoxin at a dose of 2.8 µg/mL, as determined in the first assay. In this experiment, cells were also incubated with increasing doses of FOS (75, 100, 150, 200, 300, 400 and 550 µg/mL). Untreated, control cell cultures (incubated with MEM), were included in all experiments. Cell cultures were observed under inverted microscope at 40X.

Determination of apoptosis in cell cultures

To determine whether DON induces apoptosis in the different cell lines, a third experiment was performed.

Determination of nuclear changes by DAPI (4',6-diamino-2-fenilindol)

DAPI is a nuclear stain used to evaluate morphological changes which can be visualized during cell death (Atale et al., 2014). For this experiment, HEp-2, MDBK, BT and BHK cells were seeded in 6 well-plates containing coverslips in each well for cell adherence. After 24 h, cell monolayers were treated with DON (2.8 µg/mL) and with DON and FOS (2.8 µg/mL and 550 µg/mL, respectively), according to the doses of mycotoxin and antibiotic previously determined. Cells incubated with MEM were used as untreated control. After incubation, MEM was removed; cells were washed with PBS and fixed with 4% paraformaldehyde. To evaluate the presence of nuclear morphological changes representative of apoptosis, fixed cells were stained with DAPI and then visualized under immunofluorescence microscope. The percentage of cells with altered nuclear morphology was determined for each experiment.

Detection of apoptotic cells by TUNEL (Terminal dUTP Nick End-Labeling): TUNEL labels apoptotic cells in which the DNA has been cleaved (Gavrieli et al., 1992). Thus, the TUNEL assay (DeadEnd Fluorometric TUNEL System, Promega) was used to confirm apoptosis. The procedure was performed according to the manufacturer's instructions. Cells were seeded on coverslips and treated as described for DAPI staining. Cells were fixed with 1% paraformaldehyde.

Statistical analysis: Percentages of apoptotic cells were evaluated by ANOVA/Tukey test. Statistical significance was considered when $p \leq 0.01$.

RESULTS

HEp-2 cells were incubated with 1.4 and 2.8 µg/mL of DON or left untreated as negative control cells. Both doses of DON produced cytopathic effect (CPE) characterized by cell detachment, cytoplasmic 'dots' (sign of intoxication) and loss of cell membrane. The CPE observed on cultures treated with 1.4 µg/mL was not as evident as the CPE observed when cells were incubated with 2.8 µg/mL, since signs of toxicity (globoid cells and changes in the cytoplasmic membrane) were present in absence of cell detachment (Fig. 1). In contrast, cell cultures treated with 0.35 and 0.7 µg/mL of DON showed no CPE and the cell monolayer was intact. As expected, cytopathic changes were not observed in untreated cells (Fig.2).

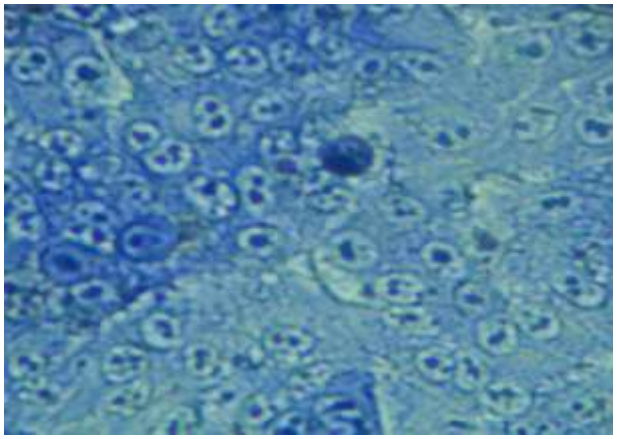


Fig. 1. Cytopathic effect of a toxic dose of DON in the absence of FOS. HEp-2 cells incubated with 2.8 µg/mL of DON in the absence of FOS (40X - Giemsa stain).

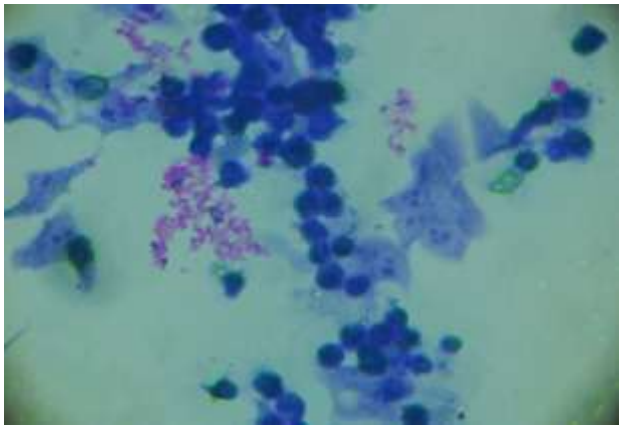


Fig. 2. HEp-2 control cell cultures. Untreated HEp-2 cells (40X - Giemsa stain).

In the second trial, cell cultures incubated with 550 µg/mL FOS (in the absence of DON) were similar to the control cells cultures, with preservation of the cell monolayer and without cell damage. The protective effect of FOS on cells treated with 2.8 µg/mL of DON was achieved at doses higher than 200 µg/mL. Concentrations of 75, 100 and 150 µg/mL showed CPE and the presence of cells with cytoplasmic dots, signs which are similar to those produced by the 2.8 µg/mL DON on HEp-2 cells. When 200, 300 and 400 µg/mL of FOS were used only 20%, 50% and 70% of the cell monolayer remained intact, respectively. A 100% protection was achieved when cells were incubated with 550 µg/mL of FOS (Fig. 3).

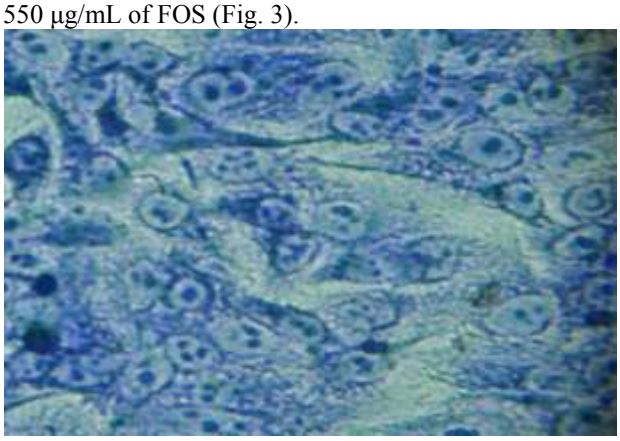


Fig. 3. HEp-2 cells incubated with a toxic dose of DON in the presence of the antibiotic. HEp-2 cells incubated with 2.8 µg/mL of DON and 550 µg/mL of FOS (40X - Giemsa stain).

On the third experiment, the different cell lines incubated with 2.8 µg/mL DON and 550 µg/mL FOS were similar to control cells (without antibiotic or toxin; Figs. 4a to d) showing the absence of cytotoxicity (Figs. 5a to d). When HEp-2, MDBK and BT cells were incubated with DON in the absence of FOS, nuclear morphological changes (nuclear condensation and chromatin fragmentation) representative of apoptosis were observed (Figs. 6a to c). For the three epithelial cell lines assayed, the percentages of cells with nuclear changes was significantly higher ($p<0.01$) for cells treated with DON (HEp-2: 5.64 $\pm$ 0.27; MDBK: 10.00 $\pm$ 0.00; BT: 51.66 $\pm$ 0.33) than for cells incubated with both the mycotoxin and the antibiotic (0.65 $\pm$ 0.50; 0.18 $\pm$ 0.00; 0.58 $\pm$ 0.05, respectively). On BHK cells, a cell line of fibroblastic origin, statistical significant differences were not detected ($p>0.01$) between cell cultures incubated with 2.8 µg/mL DON and with 2.8 µg/mL DON with 550 µg/mL FOS (0.63 $\pm$ 0.33 vs. 0.55 $\pm$ 0.05) (Fig. 6d). Fig. 7 shows the percentage of cell with altered nuclear morphology on cultures treated with DON (2.8 µg/mL) and with DON (2.8 µg/mL) and FOS (550 µg/mL).

To confirm whether the nuclear changes in the presence of DON were due to the induction of apoptotic cellular death and to establish whether FOS protected cells from this event, the TUNEL assay was performed. As shown in Fig. 4, 5 and 6, cells with fragmented DNA were detected in all cell lines exposed to DON, while apoptotic cells were not detected in the presence of FOS.

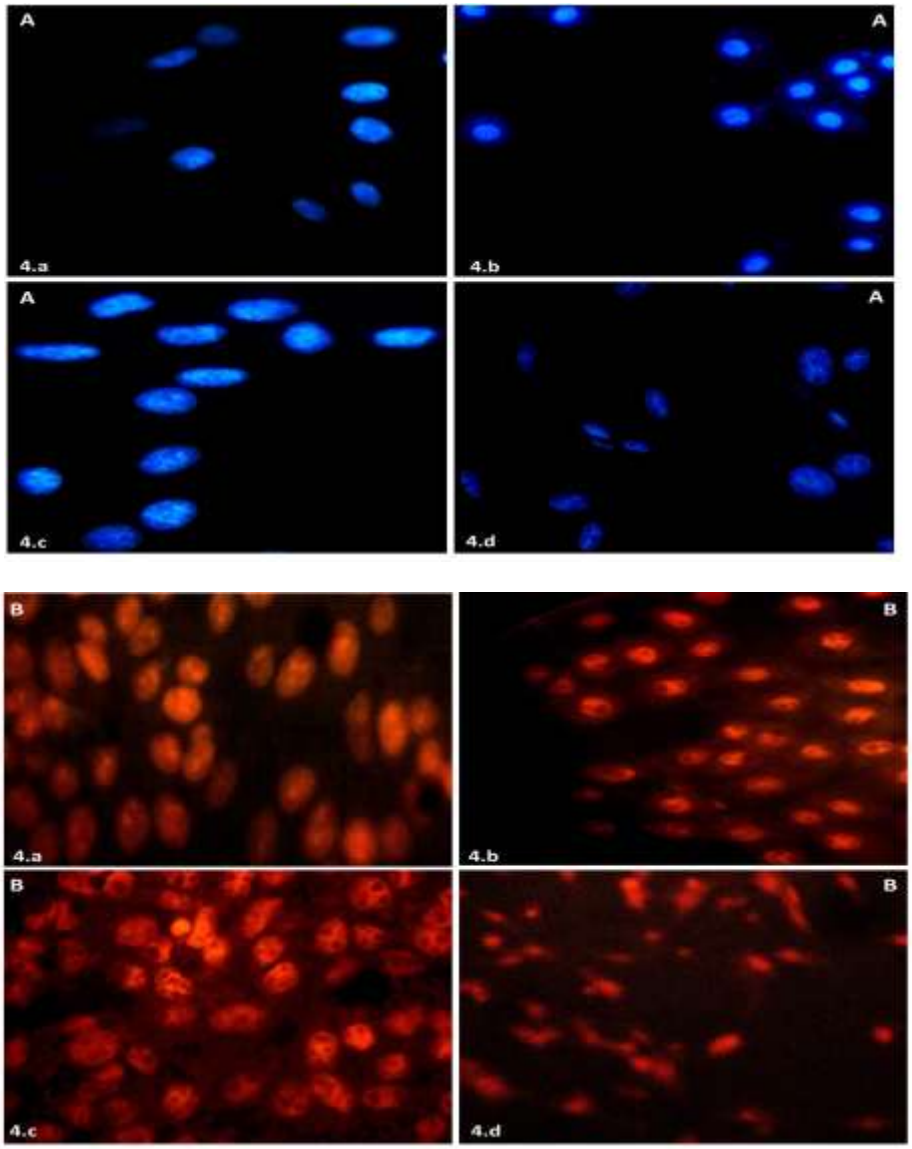
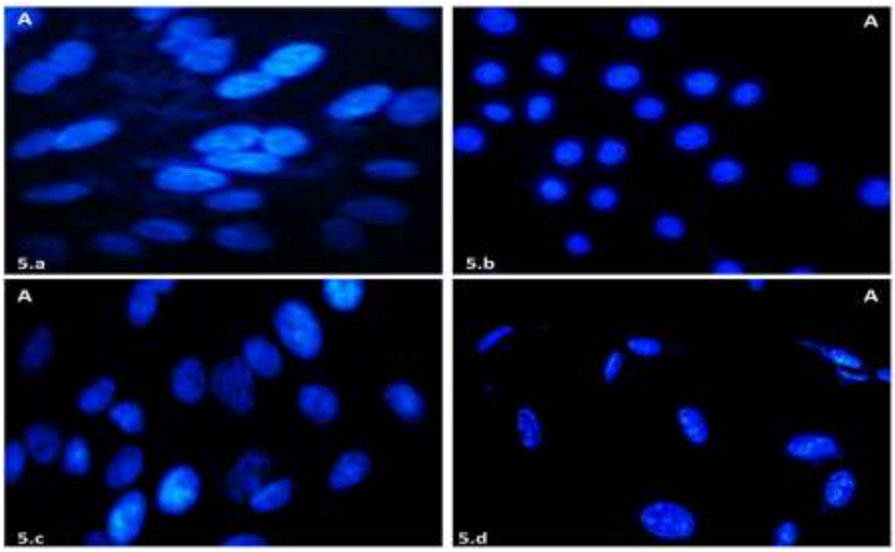


Fig. 4. Cell cultures incubated without DON and FOS Panel A) DAPI; Panel B) TUNEL a. HEp-2; b. MDBK; c. BT; d. BHK (40X).



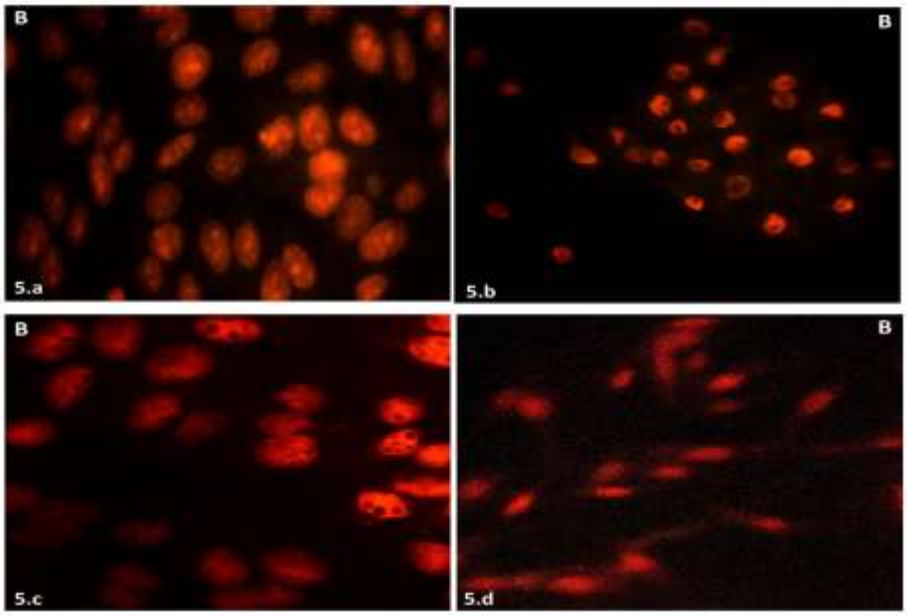
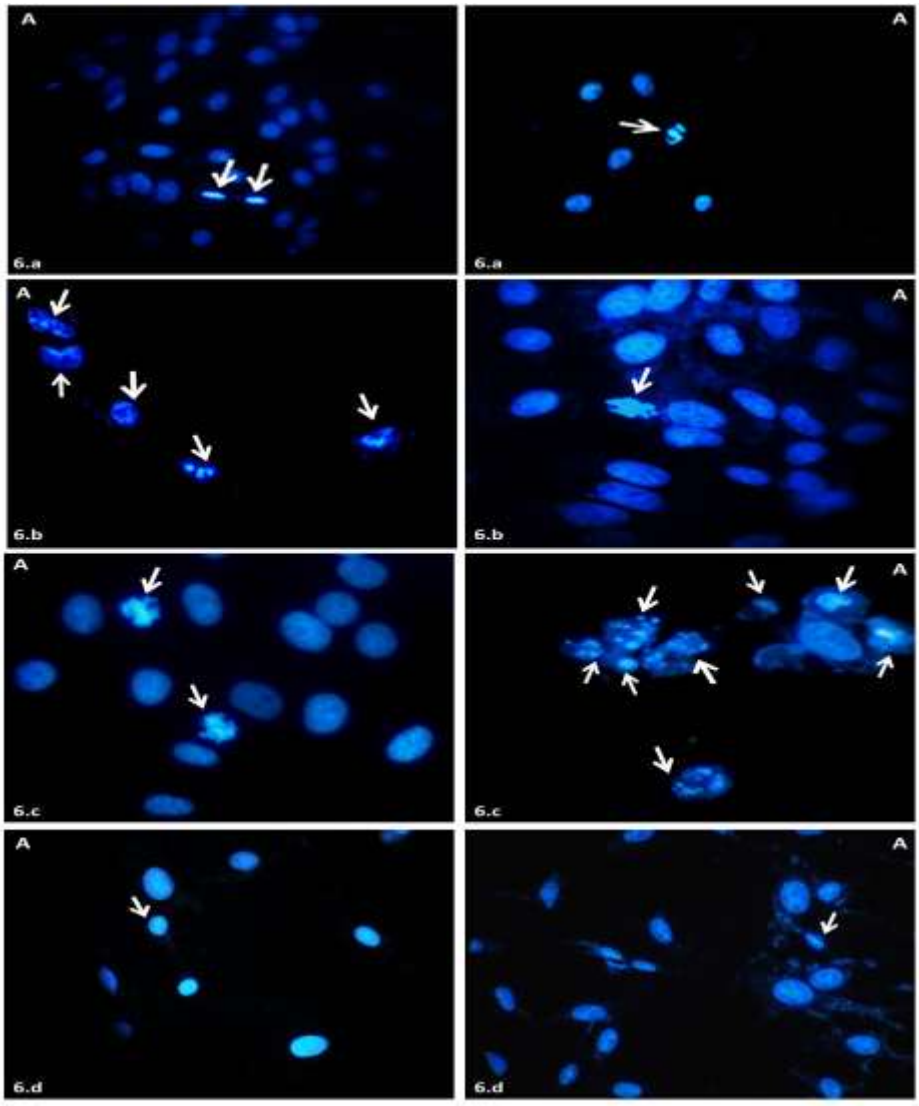


Fig. 5. Cell cultures incubated with a toxic dose of DON and a protective dose of the antibiotic. a. HEp-2; b. MDBK; c. BT; d. BHK. Panel A) DAPI; Panel B) TUNEL. Cell cultures were incubated with 2.8 µg/mL DON and 550 µg/mL FOS. (40X).



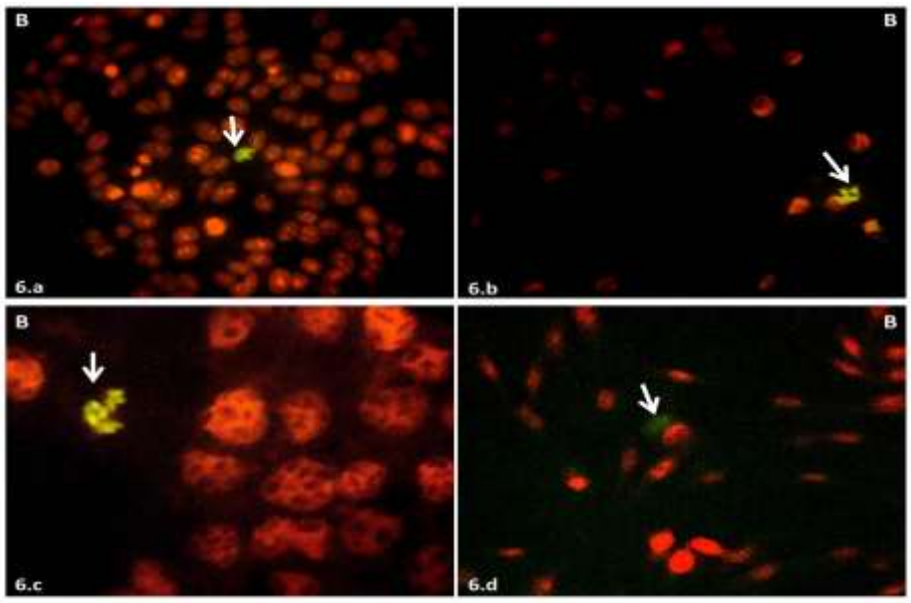


Fig. 6. Induction of apoptosis by DON treatment of cell cultures in the absence of FOS a. HEp-2; b. MDBK; c. BT; d. BHK. Panel A) DAPI staining. Nuclear morphological changes representative of apoptosis on cells incubated in the absence of FOS and Panel B) TUNEL. Detection of cells with fragmented DNA. Arrows indicate cells with nuclear changes (A) and apoptotic cells (B) (40X).

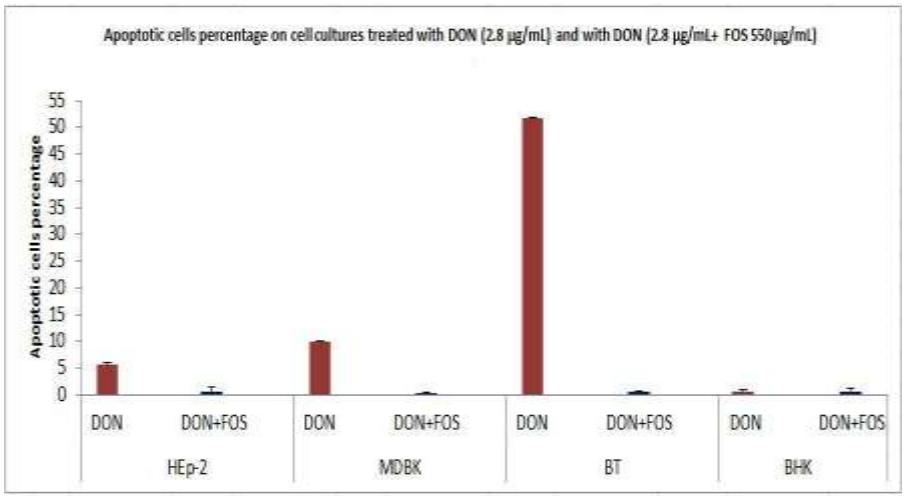


Fig. 7. Percentage of cells with nuclear changes on cultures treated with DON (2.8 µg/mL) and with DON (2.8 µg/mL) and FOS (550 µg/mL).

DISCUSSION

In this study, the effect of FOS on the cell toxicity induced by DON was analysed. Toxic effects on HEp-2 cell cultures were observed with doses of 1.4 µg/mL and 2.8 µg/mL of DON. Nevertheless, cells treated with the higher dose of the mycotoxin revealed a more evident presence of globoid cells and cytoplasmic membrane changes. Complete protection was achieved when cells were incubated with 550 µg/mL of FOS. Thus, it is evident that increasing doses of FOS protects cells against a toxic dose of DON.

Further research was conducted to determine the mechanism/s that could be involved in the cell protection achieved by the use of FOS. Inouye et al. (1982) have demonstrated in a murine model that FOS protects the proximal tubular lysosomes in the kidneys from the injury by aminoglycosides. This was evidenced *in vivo*, by suppression of myeloid body formation and protection of the integrity of lysosomal membranes in the rats treated with dibekacin. Furthermore, they have shown that, *in vitro*, there is a dose-dependent protection of the lysosomal membrane integrity of the kidney cells. A study of structure-protective activity of FOS revealed that a phosphonate anion possessing an epoxy function was important for protection and that this mechanism was different from its antibacterial mechanism. The protective effect of FOS against aminoglycoside-induced ototoxicity was also studied in rats and it was concluded that the mechanism of reduced ototoxicity was associated with the ability of FOS to inhibit the accumulation of dibekacin in the kidney and to reduce its concentration in the kidney and serum. Consequently, the amounts of dibekacin reaching the inner ear are decreased, and

ototoxicity is reduced (Ohtani et al., 1985). It has been shown that FOS also inhibits the gentamicin-induced lipid peroxidation by depressing iron release from mitochondria. It is likely that this is one of the mechanisms of FOS to protect against gentamicin-induced nephrotoxicity (Yanagida et al., 2004).

As evidenced by the results of our study, it is possible to hypothesize that another mechanism might contribute to the protection achieved by FOS. Yingjun et al. (2008) also suggested that FOS may have additional or indirect mechanisms of protection, such as that observed in the prevention of cisplatin-induced hepatotoxicity, when FOS is used in combination with other agents like selenium, methionine, taurine and zinc. All these studies are coincident in that the exact mechanism of FOS protection is not clear. However, stabilization of lysosomal membranes, interference with cellular uptake, and blockage of reactive sites on cell membranes are possible actions (Weitman et al., 1991). On the other hand, studies on FOS penetration on HEp-2 cells (Martinez et al., 2011) and pig intestinal cells (IPEC-J2) (Martinez et al., 2013) demonstrated that at no lethal doses, the mycotoxin DON does not alter the penetration of FOS on HEp-2 cells. However, they found that the non-toxic concentration of DON on IPEC-J2 cells interferes with the pharmacokinetics of the antibiotic, which reaches lower intracellular concentrations. Although it has not been demonstrated, the authors suggest that DON modifies the sodium-ion-dependent phosphate transport which FOS uses to enter the cells. Therefore, the lower intracellular concentration of FOS on IPEC-J2 cells in the presence of the mycotoxin could be attributed to the effect of DON on transport mechanisms, especially considering that it has been shown that DON affects the activities of the sodium-dependent glucose/galactose transporter (SGLT1), an intestinal transporter (Awad et al., 2007).

The results from the present work provide additional evidence demonstrating that DON induces nuclear changes in cell cultures which are indicative of apoptosis. Furthermore, the mycotoxin was able to induce apoptotic changes in all the cell lines assayed. Particularly, these changes are remarkable on cell lines of epithelial origin. This was expected since DON has proven cytotoxicity on tissues of rapid growth and fast turnover (Eriksen, 2003; Desjardins, 2006), as the epithelia. The results of this study reveal that in BT cells DON induces a higher percentage of apoptosis. Thus, the findings of this study demonstrate that certain cell types are more prone to cell death in the presence of DON. These results are in agreement with other *in vivo* (Awad et al., 2014) and *in vitro* (Katika et al., 2015) experiments showing that some cells are particularly sensitive to DON. Since the ribosome is the primary molecular target for trichothecenes, included DON, translational inhibition is an obvious mechanism of toxicity (Ueno, 1984). DON and other ribosome-binding translational inhibitors can rapidly activate mitogen-activated protein kinases (MAPKs), which are crucial for signal transduction in the immune response (Dong et al., 2002) and modulate numerous physiological processes including cell growth, differentiation and apoptosis (Cobb, 1999). The process by which trichotecenes activate MAPKs is termed the 'ribotoxic stress response' (Iordanov et al., 1997; Laskin et al., 2002). However, the exact mechanisms through which DON damages the DNA and promotes cellular toxicity are not yet well-established (Singh et al., 2015). The precise pathway that leads to the protection achieved by FOS on DON-mediated cellular damage is still unknown. However, it is evident from this study that the antibiotic is able to prevent the nuclear changes indicative of programmed cell death induced by DON.

This study is an initial step in understanding these mechanisms and it reveals that the level of induction of apoptosis appears to be dependent on the cell type. Further studies are needed to understand the implications of this effect under *in vivo* conditions and to confirm that the protective effect of the antibiotic occurs in different tissues. This is important, particularly on intestinal cells, since the mycotoxin and the antibiotic have proven effects on this kind of cells-and on immune cells, where both molecules have also been shown to modulate their functions.

CONCLUSIONS

The results from this study demonstrated that DON induces apoptosis in different cell lines and that FOS can protect cells from DNA damage. Future investigations to determine the exact way in which FOS prevents DON-induced apoptosis may be of significance, especially when considering that the mycotoxin is responsible for a ribotoxic stress response and that the antibiotic could be acting by preventing DON effects on MAPKs or other signalling pathways that trigger apoptotic death.

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REFERENCES

1. Aramayona JJ, Bregante MA, Solans C, Rueda S, Fraile LJ, García MA. Pharmacokinetics of fosfomycin in chickens after a single intravenous dose and tissue levels following chronic oral administration. Vet Res, 1997; 6: 581-588.
2. Atale N, Gupta S, Yadav UC, Rani V. Cell-death assessment by fluorescent and nonfluorescent cytosolic and nuclear staining techniques. J Microsc, 2014; 255 (1): 7-19.
3. Awad WA, Aschenbach JR, Setyabudi FMCS, Razzazi-Fazeli E, Böhm J In vitro effects of Deoxynivalenol on small intestinal D -glucose uptake and absorption of Deoxynivalenol across the

isolated jejunal epithelium of laying hens. Poult Sci, 2007; 86: 15-20.
4. Awad WA, Ghareeb K, Dadak A, Hess M, Böhm J. Single and combined effects of deoxynivalenol mycotoxin and a microbial feed additive on lymphocyte DNA damage and oxidative stress in broiler chickens. PLoS One, 2014; 31 9(1): e88028.
5. Borsa F, Leroy A, Fillastre JP, Godin M, Moulin B. Comparative pharmacokinetics of tromethamine fosfomycin and calcium fosfomycin in young and elderly adults. Antimicr Agents Chemother, 1988; 32(6): 938-941.
6. Carramiñana JJ, Rota C, Agustín I, Herrera A. Vet Microbiol, 2004; 104(1-2): 133-139.
7. Cobb MH. MAP kinase pathways. Prog Biophys Mol Biol. 1999; 71(3-4): 479-500.
8. Desjardins, AE. Fusarium mycotoxins. Chemistry, genetics and biology. The American Phytopathological Society Press, 2006; USA.
9. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. Annu Rev Immunol 2002; 20: 55-72.
10. Eriksen GS. Metabolism and toxicity of trichothecenes. Doctoral thesis Swedish University of Agriculture Science, 2003; Upsala.
11. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol, 1992; 119: 493-501.
12. Gallego A, Rodríguez A, Mata JM. Fosfomycin: pharmacological studies. Drugs Tod, 1974; 10: 161-168.
13. Gobernado M. Fosfomicina. Rev Esp Quimioter, 2003; 16(1): 15-40.
14. Inouye S, Niizato T, Komiya I, Yuda Y, Yamada Y. Mode of protective action of fosfomycin against dibekacin-induced nephrotoxicity in the dehydrated rats. J of Pharmacobiodyn, 1982; 5(12): 941-950.
15. Iordanov MS, Pribnow D, Magun JL, Dinh TH, Pearson JA, Chen SL, Magun BE. Ribotoxic stress response: activation of the stress-activated protein kinase JNK1 by inhibitors of the peptidyl transferase reaction and by sequence-specific RNA damage to the alpha-sarcin/ricin loop in the 28S rRNA. Mol Cell Biol, 1997; 17(6): 3373-3381.
16. Kahan FM, Kahan JS, Cassidy PJ, Kropp H. The mechanism of action of fosfomycin (phosphonomycin). Ann N Y Acad Sci, 1974; 235: 364-386.
17. Katika MR, Hendriksen PJ, van Loveren H, Peijnenburg A. Characterization of the modes of action of deoxynivalenol (DON) in the human Jurkat T-cell line. J Immunotox, 2015; 12(3): 206-216.
18. Laskin JD, Heck DE, Laskin DL. The ribotoxic stress response as a potential mechanism for MAP kinase activation in xenobiotic toxicity. Toxicol Sci, 2002; 69(2): 289-291.
19. Martínez G, Soraci AL, Tapia M.O. Penetración de fosfomicina en células HEp-2 y su interacción con deoxinivalenol. Anal Vet, 2011; 31(2): 23-27.
20. Mata J, Rodriguez A, Gallego A. Fosfomycin: in Vitro activity. Chemother, 1977; 23(l): 23-24.
21. Ohtani I, Ohtsuki K, Aikawa T, Sato Y, Anzai T, Ouchi J. Protective effect of fosfomycin against aminoglycoside ototoxicity. ORL, 1985; 47: 42-48.
22. Patel SS, Balfour JA, Bryson HM. Fosfomycin tromethamine. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy as a single-dose oral treatment for acute uncomplicated lower urinary tract infections. Drugs, 1997; 53(4): 637-656.
23. Pérez DS, Soraci AL, Tapia MO. In vitro penetration of fosfomycin in respiratory cells. The Pig J, 2012; 67: 1-25.
24. Petska JJ. Mechanisms of deoxynivalenol-induced gene expression and apoptosis. Food Add Contam: Part A. Special Issue: Proceedings of the 10th UJNR International Symposium on Toxic Microorganisms, 2008; 25(9): 1128-1140.
25. Popovic M, Steinort D, Pillai S, Joukhadar C. Fosfomycin: an old, new friend? Europ J Clin Microbiol Infect Dis, 2009; 29(2): 127-142.
26. Rotter BA, Thompson BK, Prelusky DB, Trenholm HL, Stewart B, Miller JD. Response of Growing Swine to Dietary Exposure to Pure Fumonisin B1 during an eight-week period: Growth and Clinical Parameters. Nat Tox, 1996; 4(1): 42-50.
27. Savard C, Gagnon CA, Chorfi Y. Deoxynivalenol (DON) naturally contaminated feed impairs the immune response induced by porcine reproductive and respiratory syndrome virus (PRRSV) live attenuated vaccine. Vacc, 2015; S0264-410X (15) 00870-1.
28. Singh S, Banerjee S, Chattopadhyay P, Borthakur SK, Veer V. Deoxynivalenol induces cytotoxicity and genotoxicity in animal primary cell culture. Toxic Mech and Meth, 2015; 25(3): 184-191.
29. Ueno Y. Toxicological features of T-2 toxin and related trichothecenes. Fundam Appl Toxicol, 1984; 4(2 Pt 2): S124-S132.
30. Weitman SD, Kamen BA. Fosfomycin-Friend or Foe? Pediatric Hematol and Oncolol, 1991; 8(3): vii-viii.
31. Yanagida, C, Ito K, Komiya I, Horie T. Protective effect of fosfomycin on gentamicin-induced lipid peroxidation of rat renal tissue. Chem and Biol Interact, 2004; 20: 148(3): 139-147.
32. Yingjun L, Xiuqiang L, Chunwei L, Gexin L, Yaping J, Hao T. Selection of agents for prevention of cisplatin-induced hepatotoxicity. Pharmacol Res, 2008; 57: 125-131.