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ABSTRACT

Immune effects of four Fusarium-toxins (FB_1, ZEA, NIV, DON) on the proliferation of Jurkat cells and porcine lymphocytes: *in vitro* study

L. Severino · R. Russo · D. Luongo · R. De Luna · R. Ciarcia · M. Rossi

Keywords Immune system · Jurkat cells · Mycotoxins · Porcine lymphocytes

Abbreviations

ConA	concanavalin A
DON	deoxynivalenol
FB_1	fumonisin B_1
FBS	foetal bovine serum
NIV	nivalenol
ZEA	zearalenol

Introduction

Fusarium toxins are secondary metabolites produced by fungi belonging to *Fusarium spp.*, commonly found as contaminants in products of vegetable origin, particularly in cereal

L. Severino · R. Russo · D. Luongo
Department of Pathology and Animal Health, Faculty of Veterinary Medicine, University of Naples Federico II, Naples, Italy

D. Luongo · M. Rossi
Institute of Food Science, ISA-CNR, Avellino, Italy

R. De Luna
Department of Public Health, ASL/AV2, Avellino, Italy

R. Ciarcia
Department of Structures, Functions and Biological Technologies, Faculty of Veterinary Medicine, University of Naples Federico II, Naples, Italy

L. Severino (✉)
Dipartimento di Patologia e Sanità Animale, Università degli Studi di Napoli Federico II, via F. Delpino 1, 80137 Napoli, Italy
e-mail: lorella.severino@unina.it

grains, in regions with temperate climate in Europe, America and Asia. Numerous toxic effects are attributed to mycotoxins both in humans and animals, such as mutagenic, cencerogenic and teratogenic properties (Hussein and Brasel 2001). Moreover, some of them can alter normal immune responses when they are present in food at levels lower than those necessary to cause the symptoms of mycotoxicosis (Oswald et al. 2005).

In the present work, we evaluated the immunomodulatory effects of four *Fusarium* toxins (FB_1, ZEA, NIV, DON) using two different experimental models: Jurkat cells and porcine lymphocytes. In addition to the activity of single mycotoxins, we evaluated possible interactions between *Fusarium* toxins to reproduce experimental conditions *in vitro* as near as possible to field conditions.

Materials and methods

Jurkat cells (human lymphoblastoid T cell line) were cultured under standard experimental conditions using RPMI-1640 medium supplemented with heat-inactivated foetal bovine serum (FBS). As our second cellular model, we utilized lymphocytes of swine obtained from whole blood collected from *vena cava cranialis* of 26 Norwegian Landrace pigs (age: 3–5 months), in heparinized sterile Vacutainer™-tubes. For the proliferation test, Jurkat cells (2×10^4 cells/well) were incubated for 24 hours with increasing concentrations of *Fusarium* toxins, objects of the current study. Initially, blood samples were diluted (1:20) with RPMI-1640 supplemented with heat-inactivated swine serum and then incubated for 72 hours with mitogen (ConA) and with increasing concentrations of mycotoxins. In both cellular models, [^{3}H]-thymidine was added to the cell culture in the last 16 hours of the incubation period; then [^{3}H]-thymidine incorporation in neo-synthetized DNA was assessed by β-counter. Results were statistically elaborated using Student *t*-test; statistical significance was reached when *P* values were below 0.05.

Results

Jurkat cells: proliferation analysis showed that FB_1 stimulated cellular proliferation, while ZEA produced an inhibitory effect. In co-incubation, the inductive effect exerted by FB_1 was completely deleted by the inhibitory effect induced by ZEA. Moreover, combination of increasing concentrations of FB_1 with constant concentrations of ZEA produced a progressive and statistically significant reduction of cellular proliferation. Similarly, incubation of cells with increasing concentrations of NIV and DON revealed an inhibitory effect on cellular proliferation for both trichothecenes; this effect was statistically significant at very low concentrations (1 μM for DON and 0.5 μM for NIV). In co-incubation, further inhibition of cellular proliferation was found.

Porcine lymphocytes: analogous results were obtained on incubating porcine lymphocytes with increasing concentrations of mycotoxins, singly and in combination. In fact, while FB_1 seemed to have no statistically significant effect on lymphocyte proliferation, increasing concentrations of ZEA induced a strong inhibitory effect from a concentration of 5 μM. In addition, NIV and DON inhibited lymphocyte proliferation from a concentration of 0.125 μM for NIV and 0.25 μM for DON. In co-incubation of cells with a FB_1 + ZEA combination, an inhibitory effect on cellular proliferation higher than that induced by ZEA alone was observed. Similarly, enhancement of inhibition of cellular proliferation was found in co-incubation with NIV + DON.

Discussion

Results obtained in the present work revealed the immunomodulatory properties of the mycotoxin objects of the current study. The combination of FB_1 + ZEA causes an evident inhibition of cellular proliferation in both cellular models utilized; hence FB_1 cannot obstruct the inhibitory effect induced by ZEA but, on increasing concentrations of FB_1, the activity of ZEA is enhanced, leading us to suppose that the actions of these mycotoxins follow different, non-competitive cellular pathways. In addition, NIV and DON inhibit cellular proliferation at very low concentrations, lower than concentrations of ZEA, in both experimental models utilized in the current study; particularly our results showed a higher toxicity of NIV than DON, in agreement with data from the literature (Rocha et al. 2005; Nasri et al. 2006). Following incubation of cells with a mixture of both trichothecenes, further inhibition of cellular proliferation was observed which is assumed to be an interaction between these mycotoxins. These results, practically reproducible in both experimental models, confirm the validity of the cellular systems utilized in the present study underlining, moreover, the utility of such systems for the study of immunotoxicity *in vitro*. They also underline the interest in studying possible interactions among different mycotoxins, particularly among those mainly present in food such as *Fusarium* mycotoxins, not only regarding their toxicodinamic aspect but also to define tolerable maximum levels of *Fusarium* toxins in food.

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References

Hussein H.S. and Brasel J.M., 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*, **167**, 101–134.

Nasri T., Bosch R.R., Voorde S., Fink-Gremmels J., 2006. Differential induction of apoptosis by type A and B trichothecenes in Jurkat T-lymphocytes. *Toxicology in Vitro*, **20**, 832–840.

Oswald I.P., Marin D.E., Bouchet S., Pinton P., Taranu I., Accensi F., 2005. Immunotoxicological risk of mycotoxins for domestic animals. *Food and Additives Contaminants*, **22**, 354–360.

Rocha O., Ansari K., Doohan F.M., 2005. Effects of trichothecene mycotoxins on eukaryotic cells: a review. *Food and Additives Contaminants*, **22**(4), 369–378.