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Effect of the *Fusarium* toxin deoxynivalenol (DON) on IgA, IgM and IgG concentrations and proliferation of porcine blood lymphocytes

Tanja Goyarts [a,*], Sven Dänicke [a], Ute Tiemann [b], Hermann-Josef Rothkötter [c]

[a] *Institute of Animal Nutrition, Federal Agricultural Research Centre (FAL), Bundesallee 50, 38116 Braunschweig, Germany*
[b] *Research Unit Reproductive Biology, Research Institute for the Biology of Farm Animals (FBN), Dummerstorf, Germany*
[c] *Institute of Anatomy, Otto-von-Guericke-University, Leipziger Strasse 44, Magdeburg, Germany*

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Abstract

An important effect of the trichothecene mycotoxins is the impairment of the immune function, but immunotoxicity studies have mainly been conducted on the mouse model. In the present study, the effect of deoxynivalenol (DON) on the proliferation of ConA stimulated porcine peripheral blood lymphocytes (PBL) was assessed in vitro after adding of 70–560 ng DON per ml medium, and in vivo after chronic and acute (one single dose) dietary DON exposure (5.7 mg/kg). Immunoglobulin (IgA, IgG, IgM) concentrations were measured by ELISA in supernatants and serum of pigs. The proliferation rate was estimated with two different assays (BrdU incorporation and MTT cleavage). In vitro the ConA stimulated proliferation was inhibited to 50% (IC_{50}) at 200 and 309 ng DON/ml for the BrdU and MTT assay, respectively, indicating a higher sensitivity of DNA synthesis to DON. Immunoglobulin concentrations in the supernatant after in vitro proliferation of PBL with increasing DON concentrations for 72 h were significantly decreased, with IC_{50} values of 120.6, 84.1 and 71.7 ng DON/ml for IgA, IgM and IgG, respectively.

In vivo significant inhibition of lymphocyte proliferation was observed only in the DON acute group using the MTT assay, but values tended to be decreased in the BrdU assay and after chronic DON exposure. Immunoglobulins (IgA, IgM and IgG) in the supernatant of cultured lymphocytes were not significantly affected after dietary DON exposure. Serum IgA of pigs showed no significant differences between the groups, whereas IgM and IgG were significant increased in the DON acute group.

Keywords: Deoxynivalenol; Ex vivo; Immunoglobulin; In vitro; In vivo; Lymphocytes; Pig; Proliferation

1. Introduction

Deoxynivalenol (DON), a trichothecene mycotoxin produced by *Fusarium* fungi, is frequently found as a contaminant in cereal grains (Rotter et al., 1996; Placinta et al., 1999). Among farm animals swine are regarded to be most susceptible to DON because of reduced feed intake and decreased weight gain already at lower dose levels (Rotter et al., 1996). However, exposure to DON and other trichothecenes results not only in economical losses due to worse performance, but also in an alteration of immune cell function, dysregulation of the humoral immune response and impairment of host resistance to pathogens (Rotter et al., 1996). Mitogen-induced proliferation, a common technique to assess immunotoxicity, was observed to be impaired or enhanced (at low concentrations) after in vitro exposure of human and murine lymphocytes to DON and other trichothecenes (Bondy and Pestka, 2000; Rotter et al., 1996). DON was also shown to affect the humoral immunity (Bondy and Pestka, 2000). IgA, IgM and IgG secretion was significantly impaired in murine lymphocyte cultures exposed to DON in vitro (Warner et al., 1994). Pestka (2003) reported that chronic exposure to DON up-regulates serum immunoglobulin A (IgA) in mice, whereas

* Corresponding author. Tel.: +49 531 5963151; fax: +49 531 5963199.
E-mail address: tanja.goyarts@fal.de (T. Goyarts).

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IgM and IgG levels were decreased. Immunoglobulin disturbances as well as the effects on proliferative response of lymphocytes caused by DON were mainly studied in mice, with comparatively few investigations on possible effects on humans or domestic animals. Understanding the basis for the immuno-dysfunction attributable to DON in pigs is also important in understanding whether human exposure to DON may have unfavourable effects, because swine are physiologically quite similar to humans (Tumbleson and Schook, 1996) and are widely used as models for human disease.

The purpose of the present study was to examine the alteration of immune function in pigs by DON using two different in vitro proliferation assays (BrdU and MTT assay) as well as the measurement of immunoglobulins (IgA, IgM and IgG). For this reason, different concentrations of pure DON were tested in vitro on mitogen stimulated porcine peripheral blood lymphocytes (PBL) derived from untreated pigs. To take into account that the effects of trichothecenes on lymphocyte proliferation were mainly attributed to an inhibition of protein synthesis (Rotter et al., 1996), in the present study the influence of the known protein synthesis inhibitor cycloheximide (CHX) was compared to the effect of pure DON in vitro. Furthermore, it was tested whether the lymphocyte proliferation of pigs was affected by a chronic ($\geqslant$4 weeks) or acute (one single dose) dietary DON exposure from naturally with *Fusarium* contaminated wheat (in vivo study), indicating a hazard of DON on the porcine immune system. Finally, IgA, IgM and IgG levels were determined in supernatants of DON exposed cultured lymphocytes and in serum of pigs fed a DON contaminated diet.

2. Material and methods

If not otherwise stated, all chemicals were purchased from Sigma (Deisenhofen, Germany).

2.1. Sample preparation

Blood samples were drawn from the pigs by jugular venopuncture. The heparinized blood was diluted 1:1 with RPMI 1640 medium (R-8758). Peripheral blood lymphocytes (PBL) were separated from the diluted blood samples using a Ficoll (F-4375) density gradient (centrifugation 400*g* for 15 min). This procedure was described to enhance the recovery of mononuclear cells that were mostly lymphocytes, with a small amount of contamination by other cells (Bøyum, 1968). The opaque interface was washed two times (centrifugation 250*g* for 8 min) in RPMI 1640 medium supplemented with 1 M HEPES buffer (H-3784), 2 mM L-glutamine (G-6392), 5 mM mercaptoethanol (M-7522), 100 U/ml penicillin G, 0.1 mg/ml streptomycin, 0.25 μg/ml amphotericin B (ABAM, A-7292) and 5% heat inactivated foetal calf serum (FCS, Biochrom AG seromed®, Berlin, Germany). Cell viability was evaluated by the trypan blue exclusion technique and was always greater than 95%. Isolated lymphocytes were adjusted to a final concentration of 1×10^6 viable cells/ml and 100 μl of cell suspension was pipetted in quadruplicate into 96-well microtitre plates (MTP, Nunc A/S, Roskilde, Denmark, Cat. No. 167008). After addition of 50 μl mitogen (2.5 μg ConA/ml) and 50 μl toxin, suspension medium was appended to an amount that a final solution of 200 μl per well was obtained. Cell cultures were incubated at 37 °C in a humidified incubator at 5% CO_2 for 72 h, after centrifugation and collection of 100 μl supernatant, 10 μl of BrdU or MTT (5 mg/ml PBS) was added and incubated for another 4 h. A BrdU proliferation kit (Roche Diagnostic GmbH, Mannheim, Germany, Cat. No. 1647229) was performed according to the manufacturer's instructions and read by microplate photometer (Powerwave, Bio-Tek Instruments GmbH, Bad Friedrichshall, Germany) at a test wavelength of 450 nm and a reference wavelength of 690 nm. The optical density of MTT assay was measured by an ELISA reader at 570 nm after dissolving the crystalline formazan product with 100 μl of 0.01 N HCl/SDS solution.

2.2. In vitro experiments

The applicability and sensitivity of two different assays to measure effects of DON on porcine lymphocytes were compared: (1) DNA synthesis with BrdU (5-bromo-2′-deoxyuridine), which is incorporated into the DNA of proliferating cells instead of thymidine and (2) metabolic activity with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay, in which the yellow tetrazolium salt is metabolized by NAD-dependent dehydrogenase (in active mitochondria) to form a dark blue formazan product (Mosmann, 1983; Widestrand et al., 1999).

In preliminary tests, it was found that 1×10^5 cells stimulated with 2.5 μg ConA/ml were optimal to examine the effects of DON on porcine PBL (Goyarts et al., 2006).

The effect of different DON concentrations (70, 140, 280, 560 ng/ml, 76 h incubation) were tested on ConA stimulated peripheral blood lymphocytes of one pig fed an uncontaminated diet. In order to compare these effects of DON with the effect of protein synthesis inhibition the known protein synthesis inhibitor cycloheximide (CHX) was added as a positive control at 50, 100, 200, 500 and 750 ng/ml [0.18, 0.36, 0.71, 1.78, 2.67 μM] to lymphocyte cultures (76 h). The proliferation rate was measured by BrdU and MTT assays.

Furthermore, immunoglobulin concentrations were evaluated in the supernatants of ConA stimulated lymphocytes exposed to DON (70, 140, 280, 560 ng/ml) for 72 h. IgA, IgM and IgG concentrations were analysed by an indirect enzyme-linked immunosorbent assay (ELISA) according to Tiemann et al. (2006). By way of example the determination of IgA is described. The antibody and reference serum were purchased from Natu Tec-Bethyl (Frankfurt a.M., Germany). Microtitre plates (Nunc C bottom Immunoplate 96 well, Cat. No. 4466612; Nunc, Wiesbaden, Germany) were coated for 60 min at RT, with