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The *Fusarium* toxin zearalenone and deoxynivalenol affect murine splenic antioxidant functions, interferon levels, and T-cell subsets☆

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ABSTRACT

This study aimed to evaluate the effects of the *Fusarium* toxin zearalenone (ZEA) and deoxynivalenol (DON) on splenic antioxidant functions, IFN levels, and T-cell subsets in mice. Herein, 360 mice were assigned to nine groups for a 12-day study. Mice were administered an intraperitoneal injection for 4 consecutive days with different concentrations of ZEA alone, DON alone, or ZEA + DON. Spleen and blood samples were collected on days 0, 3, 5, 8, and 12. Mice in each of the experimental groups showed dysreglated splenic antioxidant functions, IFN levels, and T-cell subset frequencies, suggesting that the immune system had been affected. The ZEA + DON-treated groups, especially the group that received a higher concentration of ZEA + DON (Group D2Z2), showed more obvious effects on the dysregulation of splenic antioxidant functions, IFN levels, and T-cell subsets. This finding suggested that DON and ZEA exerted synergistic effects.

1. Introduction

The contamination of cereal grains with toxic secondary metabolites of fungi, known as mycotoxins, represents an ongoing challenge in animal nutrition as both animal health and performance may be compromised in addition to effects on the quality of animal-derived food. Both zearalenone (ZEA) and deoxynivalenol (DON) are important *Fusarium* toxins that can occur in feedstuffs and cause toxicity in farm animals (Fink-Gremmels and Malekinejad, 2007; Abid-Essefi et al., 2012; Girgis et al., 2010).

The immune system is a major target of mycotoxins. A high dose of DON (25 mg/kg body weight) was found to result in reduced *Interferon* (*IFN*)-γ and *Transforming growth factor* (*TGF*)-β mRNA transcript levels in murine Peyer's patches (Li et al., 2005). Other studies have shown that ZEA causes reduced levels of IFN-γ in aged mice (Calemine et al., 2003) and a slight reduction in both the mitotic index and survival of bovine lymphocytes (Lioi et al., 2004). The influence of *Fusarium* toxins on lymphocyte subpopulations in different animal species has also been examined. However, the results of previous studies have been inconsistent (Pestka et al., 1994, 2004; Dänicke et al., 2001; Döll et al., 2003; Levkut et al., 2011). To the best of our knowledge, only a few studies have examined the combined effects of DON and ZEA on splenic IFN levels and T-cell subsets in mice.

Previous studies have shown that both ZEA and DON can separately induce oxidative stress *in vivo* and *in vitro* (Ren et al., 2015a; Liang et al., 2015). However, the combined effects of DON and ZEA on splenic antioxidant functions were found to be limited. In this

Abbreviations: ZEA, zearalenone; DON, deoxynivalenol; MDA, malondialdehyde; OH^-, the inhibition capacities of hydroxyl free radicals; GSH-Px, glutathione peroxidase activities; SOD, superoxide dismutase; T-AOC, total antioxidant capacities; IFN, interferon; SD, standard deviation.

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present study, we evaluated the effects of the *Fusarium* toxin ZEA and/or DON on splenic antioxidant functions, IFN levels, and T-cell subsets in mice.

2. Materials and methods

2.1. Reagents

All chemicals were of the highest grade of purity commercially available. ZEA and DON were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous ethanol was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). DON was diluted in 10% ethanol to concentrations of 0.15 or 0.25 mg/mL. ZEA was diluted in 10% ethanol to concentrations of 2 or 3 mg/mL.

2.2. Animals and experimental design

Female Kunming mice (Musmusculus Km), bedding, and feed were purchased from the Laboratory Animal Centre at the West China Center of Medical Sciences, Sichuan University. Animals were acclimatized for 1 week prior to use. The temperature (21–24 °C) and relative humidity (40–55%) remained constant and room lights were maintained on a 12 h light (6:00–18:00)/12 h dark (18:00–6:00) cycle. Animals were caged using dust-free poplar chips for bedding and were fed a standard diet for rodents. Food and water were provided *ad libitum* during the acclimatization period and throughout the entire study. All mice (weight 20 ± 2 g) used in this study were healthy. All experimental procedures were in accord with the Institutional Guidelines for the Care and Use of Laboratory Animals.

A total of 360 mice were randomly divided into nine groups, which received different treatments that are shown in Table 1. From day 1, mice in all groups received continuous intraperitoneal injections for 4 days with different concentrations of ZEA alone, DON alone, or ZEA + DON every 24 h at 8:00 in the morning. Additionally, five mice were randomly selected from each group to harvest spleen samples on days 0, 3 (before the third injection), 5 (the first day after finishing the toxin injections), 8 (the fourth day after finishing the toxin injections), and 12 (the eighth day after finishing the toxin injections) of this experiment.

2.3. Sample collection

On days 0, 3, 5, 8, and 12 of this experiment, five mice were collected from each group. Animals were euthanized with overdoses of anesthetic. Blood was collected into microcentrifuge tubes by orbital vein puncture. Spleen tissues were homogenized (1:9 w/v) using a glass Teflon homogenizer (Heidolph S01 10R2RO) in 0.9% normal saline buffer. To obtain tissue supernatant for measuring antioxidant enzyme activities, MDA levels, and IFN levels, homogenates were centrifuged at 3000 × *g* for 10 min at 4 °C.

2.4. Determination of antioxidant enzyme activities and malondialdehyde content

Antioxidant enzyme detection kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Levels of malondialdehyde (MDA), the inhibition capacities of hydroxyl free radicals (OH^-), glutathione peroxidase (GSH-Px) activities, superoxide dismutase (SOD) levels, and total antioxidant (T-AOC) capacities in the spleen were determined using these kits following the manufacturer's instructions.

2.5. Determination of IFN levels

IFN detection kits were purchased from the Wuhan Colorfulgene Bioengineerning Institute (Wuhan, China). Concentrations of IFN-α, IFN-β, and IFN-γ in the spleen were determined using these kits according to the manufacturer's instructions.

2.6. T-cell subsets

The frequencies of $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ T cells in the blood were determined using flow cytometry (Beckman Coulter), as described by Ren et al. (2015b).

Approximately 1 mL of peripheral blood was collected in 5-mL heparinized vacuum tubes, mixed with an equal volume of PBS (0.01 M and pH 7.4), and carefully layered on the surface of lymphocyte separation medium. Centrifugation was carried out at 200 × *g* for 20 min at room temperature. Lymphocytes were collected, transferred to another centrifuge tube, and washed with PBS. The resulting pellet was resuspended at a concentration of 1×10^6 cells/mL in PBS. Approximately 1 mL cell suspension was transferred to another tube and centrifuged at 200 × *g* for 5 min. The supernatant was discarded. Cells were stained with 10 μL anti-mouse CD3 phytoerythrin (PE; Southern Biotechnology Associates, Birmingham, AL, USA), anti-mouse CD4 PE (Southern Biotechnology Associates), or anti-mouse CD8 PE (Southern Biotechnology Associates) for 20 min at room temperature and then were washed with PBS. The supernatant was discarded, and cells were resuspended in 0.5 mL PBS and analyzed by flow cytometry.

2.7. Statistical analysis

Excel was used for data pre-processing and statistical analyses using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). The Duncan test was used for multiple comparisons. Data were expressed as means ± SD; $P < 0.05$ or 0.01 were considered to indicate statistically significant differences.

3. Results

Changes in spleen antioxidant functions and IFN levels are shown in Tables 2–9. In all tables, the horizontal comparisons represent splenic antioxidant functions, IFN levels, and T-cell subsets in the same group of mice at different time points (represented by subscripts), and the vertical comparisons represent splenic antioxidant

Table 1
Grouping and treatment of laboratory animals.

	Grouping								
Toxins and diluent	Control group	Group D1	Group D2	Group Z1	Group Z2	Group D1Z1	Group D1Z2	Group D2Z1	Group D2Z2
DON (mg/kg BW)	–	1.5	2.5	–	–	1.5	1.5	2.5	2.5
ZEA (mg/kg BW)	–	–	–	20	30	20	30	20	30
Diluent	+ +	+	+	+	+	–	–	–	–

"–" Represents not injected with the substance; "+" represents 200 μL injected diluent; "1.5" and "2.5" represent injection of 200 μL 1.5 and 2.5 mg/kg BW deoxynivalenol, individually; "20" and "30" represent injected of 200 μL 20 and 30 mg/kg BW zearalenone, individually.