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Effect of the *Fusarium* toxins, zearalenone and deoxynivalenol, on the mouse brain

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ABSTRACT

The aim of this study was to find effects of *Fusarium* toxins on brain injury in mice. We evaluated the individual and combined effect of the *Fusarium* toxins zearalenone and deoxynivalenol on the mouse brain. We examined brain weight, protein, antioxidant indicators, and apoptosis. After 3 and 5 days of treatment, increased levels of nitric oxide, total nitric oxide synthase, hydroxyl radical scavenging, and malondialdehyde were observed in the treatment groups. This was accompanied by reduced levels of brain protein, superoxide dismutase (apart from the low-dose zearalenone groups), glutathione, glutathione peroxidase activity, and percentage of apoptotic cells. By day 12, most of these indicators had returned to control group levels. The effects of zearalenone and deoxynivalenol were dose-dependent, and were synergistic in combination. Our results suggest that brain function is affected by zearalenone and deoxynivalenol.

1. Introduction

Mycotoxins are potentially highly toxic filamentous fungi secondary metabolites that frequently contaminate agricultural commodities used as animal feed (Hussein and Brasel, 2001; Sudakin, 2003). It is estimated that approximately 25% of the world's crop production is contaminated with mycotoxins to some extent (CAST, 2003). Mycotoxin contamination is associated with a number of economic impacts, including the destruction of highly contaminated crops unsuitable for human or animal consumption, costly mycotoxin screening programs, reduced production efficiency, and impaired livestock health due to contaminated feeds (CAST, 2003). Worldwide, *Fusarium* fungi are among the most important toxin-producers associated with cereals (Ueno, 1983). These fungi produce a variety of mycotoxins, including fumonisins, fusarins, trichothecenes, zearalenone (ZEA), and T-2 toxin.

ZEA is an estrogenic mycotoxin produced by *Fusarium*. It has a similar structure to estrogen and competes with 17β-estradiol for binding to the estrogen receptor, resulting in fertility and reproductive problems (Takemura et al., 2007). Deoxynivalenol (DON), or vomitoxin, is a commonly encountered type-B trichothecene mycotoxin produced by *Fusarium* species that is found in cereals and grains. Laboratory and farm animal studies have shown that DON elicits a complex spectrum of toxic effects. Chronic exposure to low doses of DON can lead to anorexia, impaired weight gain, and immunotoxicity; acute exposure to high doses can cause diarrhea, vomiting, leukocytosis, circulatory shock, and ultimately death (Pestka and Smolinski, 2005).

The brain is an essential organ for life. It is the superior part of the central nervous system. Both ZEA and DON have been shown to produce neurological symptoms in animals. However, few studies have investigated the impact of ZEA and DON on induced brain toxicity, and these have principally investigated the effect of ZEA or DON alone (Lessard et al., 2015; Hooft et al., 2011). Only a few studies (Winkler et al., 2014; Ren et al., 2014; Seeling et al., 2005, 2006) have examined the effects of ZEA and DON on health and performance simultaneously, using blood toxin residues as a biomarker of internal exposure.

Given that ZEA and DON frequently occur together in animal feed, we believe that evaluating the combined effects of exposure to ZEA and DON simultaneously would be informative. In this study, we evaluated the effects of ZEA and DON exposure, individually and in combination, on the mouse brain. We examined brain weight, protein levels, antioxidant indicators, and the percentage of apoptotic cells.

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2. Materials and methods

2.1. Reagents

All chemicals were the highest purity grade 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 with 10% ethanol to give final concentrations of 0.15 mg/mL and 0.25 mg/mL. ZEA was diluted with 10% ethanol to give final concentrations of 2 mg/mL and 3 mg/mL.

2.2. Animals and experimental design

Female Kunming mice (*Mus Musmusculus* Km), bedding, and feed were purchased from the Laboratory Animal Center at the West China Center of Medical Sciences, Sichuan University. Animals were acclimatized for 1 week prior to use. Mice were maintained at a constant temperature (21–24 °C) and relative humidity (40–55%) with a 12-h light (6:00–18:00 h):dark (18:00–6:00 h) cycle. Animals were kept in cages with dust-free poplar chip bedding and fed a standard rodent diet. Food and water were freely provided during the acclimatization and study period. All mice used in the study were certified healthy and weight 20 ± 2 g.

The 360 mice used in this study were randomly divided into 9 groups, as shown in Table 1. The mice in each group were treated by intraperitoneal injection with ZEA alone, DON alone, or a mixture of ZEA and DON every 24 h for 4 days. Ethanol was used as a control. All experimental manipulations were performed in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals.

2.3. Sample collection

On days 3, 5 (day 1 post-treatment), 8 (day 4 post-treatment), and 12 (day 8 post-treatment), 8 mice were randomly selected from each group for brain samples. The samples harvested on day 3 were collected prior to the third injection. Animals were euthanized by anesthetic overdose. All procedures were approved by the Ethical and Animal Welfare Committee of Sichuan Province, China. Brains were dissected and weighed after drying with filter paper. Brain tissues were homogenized (1:9 w/v) using a glass Teflon homogenizer (Heidolph S01 10R2RO) in 0.9% normal saline. Antioxidant enzyme activities and malondialdehyde (MDA) levels were measured in tissue supernatants after centrifugation at 3000*g* for 10 min at 4 °C.

2.4. Determination of antioxidant enzyme activities, and protein, MDA, and neurotransmitter levels

Detection kits were purchased from the Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). The level of protein, MDA, glutathione (GSH), superoxide dismutase (SOD), and nitric oxide (NO), and the hydroxyl radical scavenging (OH), GSH peroxidase (GSH-Px), and the total nitric oxide synthase (TNOS) activities were determined in accordance with the manufacturer's instructions.

2.5. Detection of apoptosis by annexin V flow cytometry

The percentage of apoptotic cells in day 3, 5, 8, and 12 brains was determined as described by Ren et al. (2015). Approximately 100 μL of cellular suspension was stained with 5 μL of V-FITC (BD Biosciences, USA) and 5 μL of propidium iodide (5 μL/mL propidium iodide, 0.5% Triton X-100, 0.5% RNAse, phosphate buffered saline) for 15 min at room temperature in the dark. Approximately 400 μL of 1 × binding buffer was then added to each tube and flow cytometry was performed within 1 h.

2.6. Statistical analysis

Excel was used for pre-processing data and SPSS 17.0 was used for statistical analysis. Duncan's multiple range test was used for comparisons. All values are expressed as the mean ± standard deviation. A p value <0.05 or 0.01 was considered as significantly different.

3. Results

Fig. 1 indicates the changes in brain weight with treatment. The graphs show the brain weights in the same groups of mice at different time points. Brain weight significantly increased over time in all groups. In the individual graphs, the brain weight of each treatment group at the same experimental time is shown. No significant differences were observed between the groups at each time point.

Fig. 2 shows the protein content of the brains. On days 3 and 5, the protein level in the treatment groups decreased, and this effect was greater as the toxin dose increased ($p < 0.05$ or $p < 0.01$). The combined effect of DON and ZEA was significantly greater than DON or ZEA alone ($p < 0.05$ or $p < 0.01$), and was synergistic in nature. By day 8, the effect of toxin treatment on protein levels had reduced. On day 12, there were no differences between the treatment groups.

Fig. 3 shows the brain SOD levels. On days 3 and 5, the SOD level in the low-dose ZEA groups (Z1, D1Z1, and D2Z1) was significantly higher than in the control group ($p < 0.01$); the SOD level in the other treatment groups was significantly lower than in the control group ($p < 0.05$ or $p < 0.01$). By day 8, the SOD level in the low-dose ZEA groups (Z1, D1Z1, and D2Z1) had reduced and the SOD level in the other treatment groups had increased. The SOD level of all treatment groups was lower than the control group ($p < 0.05$ or $p < 0.01$) on day 8. On day 12, only the D2Z2 treatment group SOD level was significantly different to control ($p < 0.01$). The effect of all treatments was dose-dependent. The combined effect of ZEA + DON was greater than the sum of ZEA or DON alone, and was synergistic in nature ($p < 0.05$ or $p < 0.01$).

Fig. 4 shows brain GSH levels. The brain GSH levels were lower in all of the experimental groups compared with the control group ($p < 0.05$ or $p < 0.01$); this effect was dose-dependent. On days 8 and 12, the brain GSH levels in all of the experimental groups had increased, but remained lower than in the corresponding control groups ($p < 0.05$ or $p < 0.01$). The brain GSH-Px activity (Fig. 5) analysis showed a similar trend to the GSH levels. In contrast, the brain NO levels (Fig. 6), the TNOS activity (Fig. 7), hydroxyl radical scavenging activity (Fig. 8), and MDA levels (Fig. 9) had decreased by days 8 and 12. The brain MDA levels were higher in the ZEA + DON treatments groups than in the individual ZEA or DON treatment groups ($p < 0.05$ or $p < 0.01$). All of the previously stated trends showed that the effects of ZEA and DON were synergistic and dose-dependent.

Fig. 10 indicates the percentage of apoptotic brain cells in each group. On days 3 and 5, the percentage of apoptotic cells was increased in all of the experimental groups ($p < 0.05$ or $p < 0.01$). Apart from the D1 and D2 groups, in which D1 treated groups showed higher rates of apoptosis than the D2 treated groups ($p < 0.01$), the effect of treatment was dose-dependent. The percentage of apoptotic cells in the ZEA + DON groups was higher than in the Z1 or DON-alone groups ($p < 0.05$ or $p < 0.01$). These effects were also dose-dependent. Among all of the experiment groups, Z2 groups showed the greatest change ($p < 0.01$). Apart from the D2Z2