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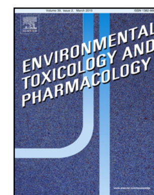

Protective role of selenium in the activities of antioxidant enzymes in piglet splenic lymphocytes exposed to deoxynivalenol

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ABSTRACT

We evaluated the effects of selenium (Se) on antioxidant enzymes of piglet splenic lymphocytes exposed to deoxynivalenol (DON). We measured cell viability, the activities of several antioxidant enzymes, and lactate dehydrogenase (LDH), as well as total antioxidant capacity (T-AOC) and the levels of malonaldehyde (MDA) and hydrogen peroxide (H_2O_2). We found that DON exposure increased the concentrations of LDH, MDA, and H_2O_2 in all experimental groups in a dose-dependent manner, while the concentrations of other antioxidant enzymes were decreased. In Se-pretreated DON-exposed cells, damage to antioxidant enzymes was reduced, especially in the lower-dose DON groups over longer exposure times. These results may indicate that in piglet splenic lymphocytes, Se can alleviate DON-induced damage to antioxidant enzymes by improving glutathione peroxidase activity. Se may function as a potential antioxidative agent to alleviate DON-induced oxidative stress.

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1. Introduction

Deoxynivalenol (DON) is a type B trichothecene that is mostly produced by *Fusarium graminearum* and *Fusarium culmorum* (Smolinsky and Pestka, 2005). A multitude of studies have shown that DON can cause a broad range of toxic effects on humans and animals, such as growth suppression, diseases of the digestive tract, and dysregulation of the immune system (Hussein and Brasel, 2001; Pestka, 2007; Pestka et al., 2008). Present methods cannot completely remove DON from feed and grain (Cheng et al., 2010). The contamination of DON has resulted huge damage in feed industry, food safety, and animal husbandry.

DON has been found to impair antioxidant enzymes and DNA and protein synthesis (Dänicke et al., 2006; Zhang et al., 2009), as well as induce apoptosis (Katika et al., 2012). However, immune organs are the main target of DON (Dong et al., 1994; Ren et al., 2003; Zhou et al., 1998). Pigs are known to be more affected by DON than mice, poultry, and ruminants (Rotter, 1996). At the cellular level, macrophages, T cells, and B cells are all highly sensitive to trichothecenes (Zhou et al., 2003). Multiple *in vitro* studies have demonstrated that DON treatment of different cells (Caco-2, U937, HEK-293, chicken embryo fibroblast DF-1) decreases the activities of intracellular antioxidant enzymes in a dose-and time-dependent manner (Costa et al., 2009; Dinu et al., 2011; Kouadio et al., 2007, 2005; Li et al., 2013). In our previous studies, DON induced apoptosis (Ren et al., 2015a) and the secretion and mRNA expression of related cytokines in chicken splenic lymphocytes in vitro (Ren et al., 2014); In addition, it caused the dysregulation of porcine splenic antioxidant functions and immunoglobulin (Ren et al., 2015b). Consequently, we sought to characterize the antioxidant enzymes induced in splenic lymphocytes of piglets.

Mycotoxin-induced damage to antioxidant enzymes can be reduced by antioxidant supplementation in an appropriate manner (Frankič et al., 2008; Guney, 2012; Rotter, 1996). Selenium (Se), as an essential micronutrient for individuals and animals, are important in redox regulation. Se can protect the antioxidant function of animals through glutathione peroxidases that remove excess

Abbreviations: AFB1, aflatoxin B1; CAT, catalase; DON, deoxynivalenol; GPx, glutathione peroxidase; GSH, glutathione; H_2O_2, hydrogen peroxide; LDH, lactate dehydrogenase lactate dehydrogenase; MDA, malondialdehyde; OTA, ochratoxin A; PBS, phosphate-buffered saline; SOD, superoxide dismutase; Se, selenium; T-AOC, total antioxidative capacity; ZEA, zearalenone.

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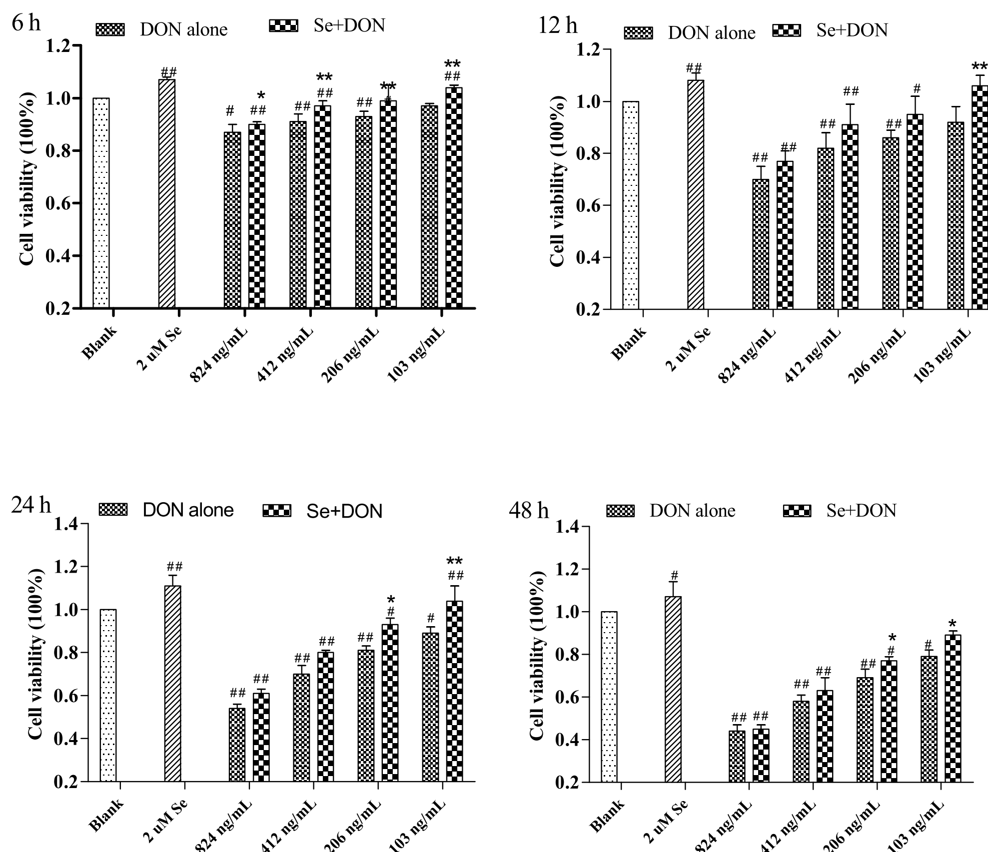

Fig. 1. Effects of Se (Na_2SeO_3, 2 μM) on DON-treated piglet splenic lymphocytes. Cells were seeded at a density of 3×10^5 cells/well in 96-well plates and treated with Na_2SeO_3 (2 μM) and various concentrations of DON, then incubated for 6, 12, 24, or 48 h. Cell viability was determined by the CCK-8 assay. Significant changes are indicated by # for $p < 0.05$ and ## for $p < 0.01$ in comparison with controls. Within the DON treatment group, significance compared with the control cells without Se treatment is indicated as * for $p < 0.05$ and ** for $p < 0.01$.

of potentially damaging radicals produced during oxidative stress (Maggini et al., 2007; Zeng, 2009). Se supplementation is indispensable to the activity of Se-dependent enzymes, such as glutathione peroxidases, thioredoxin reductases, iodothyronine deiodinases, and selenophosphate synthetases (Liu et al., 2011; Zeng, 2009). The protective action of Se against DON has been shown in male rats (Rizzo et al., 1994). The antioxidative effect of Se as sodium selenite (Na_2SeO_3) has not been studied *in vitro* in a DON-induced splenic lymphocyte culture model. Accordingly, our objective in the present study was to evaluate the protective effects of Se on DON-induced antioxidants in splenic piglet lymphocytes.

2. Materials and methods

2.1. Chemicals

Purified DON was purchased from Sigma-Aldrich (USA). Na_2SeO_3 powder was purchased from Xiya Reagent (China). Fetal bovine serum (FBS) was obtained from the HyClone (USA). Piglet lymphocyte separation medium was purchased from Tianjin Hao Yang Biological Manufacture (China). RPMI-1640 medium was purchased from Wuhan Boster (China). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo (Japan). Kits for testing glutathione (GSH), malonaldehyde (MDA), total antioxidant capacity (T-AOC), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), hydrogen peroxide (H_2O_2), and lactate dehydrogenase (LDH) in piglets were obtained from the Nanjing Jiancheng Bioengineering Institute (China).

2.2. Cell culture

All study procedures were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University. Ten 30–40-day-old healthy weaned piglets (Duroc × Large White × Landrace) were purchased from New Hope Group (China).

Piglets in good condition were anesthetized with an intramuscular injection of 846 anesthetic mixture (haloperidol, dihydroetorphine, and 2,4-dimethylaniline thiazole) using 0.8 mL/kg body weight as the dose. After laparotomy, spleen samples were removed aseptically. The spleen samples were then soaked in 75% alcohol for 5 min, and cleaned with phosphate-buffered saline (PBS) three times. After stripping the surrounding connective tissue and fat, splenic tissue was cut into suitable fragments and dispersed to a single cell suspension using a 200-mesh gauze sieve. The cell suspension was gradually added to a centrifuge tube with the same volume of lymphocyte separation medium. After centrifugation for 20 min at 400 × *g*, we collected the second layer of cells. To obtain relatively pure lymphocytes, the cell sample was washed twice with RPMI-1640 medium at 500 × g for 5 min at room temperature. More than 95% of cells were viable, based on trypan blue dye exclusion. Finally, the density of spleen lymphocytes was adjusted to 3×10^6 cells/mL (Zhuang et al., 2015), and the cells were then exposed in RPMI-1640 medium containing 10% FBS, HEPES (Wuhan Boster), 100 U/mL penicillin, and 100 μg/mL streptomycin, and incubated with 5% CO_2 at 37 °C. All samples were tested as three independent replicates. And in each index, three different samples were used.

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