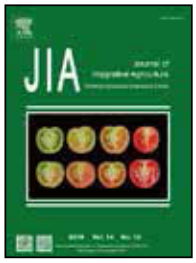
Journal of Integrative Agriculture 2015, 14(10): 2058–2064

Available online at www.sciencedirect.com

ScienceDirect



RESEARCH ARTICLE

Tissue distribution of deoxynivalenol in piglets following intravenous administration

DENG Xian-bai, DIN Huan-zhong, HUANG Xian-hui, MA Yong-jiang, FAN Xiao-long, YAN Hai-kuo, LU Pei-cheng, LI Wei-cheng, ZENG Zhen-ling

College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, P.R.China

Abstract

Contamination of deoxynivalenol (DON) in grains is common worldwide and pigs are particularly susceptible to this mycotoxin. The distribution of DON in porcine tissues following intravenous administration was investigated in this study. Fifteen pigs were randomly divided into three groups. Animals in groups A and B were administrated with DON at the dose of 250 and 750 μg kg^{-1} body weight, respectively, while group C served as blank control. Plasma, bile and 27 tissues were collected at 30 min post-administration. DON concentrations in all samples were tested using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). To observe the distribution of DON in tissues, these samples were further subjected to the immunohistochemical analyses. Totally, the bile and 13 tissues were sampled for DON-based detection, including kidney, mesenteric lymph nodes, muscle, stomach, jejunum, colon, plasma, spleen, rectum, cecum, liver, ileum, and duodenum. No significant difference was observed for the concentrations of DON in duodenum, ileum and liver samples between groups A and B; while the DON concentrations in cecum and rectum of group B were significantly higher (*P*-value <0.05) than those in group A. In addition, the DON concentrations in stomach, jejunum, colon, mesenteric lymph nodes, muscle, kidney, spleen, bile, and plasma of group B were remarkably higher than those of group A (*P*-value<0.01). Levels of DON in other 14 tissues including medulla oblongata, midbrain, diencephalon, pons, tip and tongue body, tongue, soft palate, tonsils, pharyngeal mucosa, oral buccal mucosa, thymus, thyroid, esophagus and adrenal gland were all below the limit of detection. The results of immunohistochemistry showed that 11 tissue samples (medullaoblongata, tonsil, adrenal medulla, thyroid gland, thyroid, stomach, duodenum, jejunum, kidney, spleen, and mesenteric lymph nodes) were positive and DON was mainly distributed around blood vessels in these tissues. Therefore, we believed that concentrations of DON in tissues differ when pigs are in exposure to various dosages and DON causes lesions in many pig tissues.

Keywords: deoxynivalenol, tissue distribution, immunohistochemical staining, HPLC-MS/MS, swine

1. Introduction

Deoxynivalenol (DON, vomitoxin) is a trichothecene mycotoxin produced by several plant pathogenic fungi and most commonly detected in grain-based food and small grains such as wheat, maize, barley, oats, and rice all over the world (Jelinek *et al*. 1989; Anonymous 1993;

Received 23 September, 2014 Accepted 19 January, 2015
DENG Xian-bai, E-mail: xbdeng@scau.edu.cn;
Correspondence ZENG Zhen-ling, Mobile: +86-13609768425, Fax: +86-20-85280234, E-mail: zlzeng@scau.edu.cn

doi: 1016/S2095-3119(14)60942-4

Aringoli *et al.* 2012; Rodrigues and Naehrer 2012; Streit *et al.* 2012; Lindblad *et al.* 2013). DON causes frequent, unpreventable contamination of grains and poses multiple potential health concern to human and animals (Amuzie *et al.* 2008; Shephard *et al.* 2013). Exposure to DON has brought about various adverse effects such as feed refusal, anorexia, weight loss, vomiting, and immunotoxic effects in animals (Forsyth *et al.* 1977; Young *et al.* 1983; Trenholm *et al.* 1984; Prelusky *et al.* 1994; Rotter *et al.* 1996; Li *et al.* 2013). Acute effects of DON intoxication produce symptoms of abdominal pain or a feeling of fullness in the abdomen, dizziness, headache, throat irritation, nausea, vomiting, diarrhea, and blood in stools to human (Ambrus *et al.* 2011). In addition to these hazards, DON has been suggested in association with suppression of normal immune responses to pathogens, and simultaneously inducing autoimmune-like disease, which are similar to human immunoglobulin A (IgA) nephropathy (Berger 1969; Rasooly and Pestka 1992; Pestka and Smolinski 2005).

Deoxynivalenol, fumonisin, and zearalenone are commonly detected mycotoxins in grains in China, with DON found most commonly and at the highest amount. To date, the highest level of DON detected in China was reported to be 170.22 ng kg^{-1} by Zhang *et al.* (2000), who investigated the contents of DON in wheat flour and maize in Linxian and Cixian counties (Hebei Province, China) by high performance liquid chromatography (HPLC). In addition, DON was found in 53.8 and 100% of samples from the two counties, respectively.

A risk assessment was carried out based on probable daily intake (PDI) and tolerable daily intake (TDI). Despite the average PDI of consumers was below TDI, special attention should be paid on consumers with high PDI as well as other susceptible population. Šarkanj *et al.* (2013) found that concentrations of DON and its main conjugated forms, DON-15-GlcA, exceeded the limit of detection in up to 97.5% of the urine samples, showing exceptionally high intake of DON, with a level of 3300% of the established TDI. Another study carried out by Ediage *et al.* (2013) indicated that the mean concentration of DON (3.0 ng mL^{-1}) detected in the urine of male children was significantly higher than that in female children (*P*-value was 0.021). At present, the amounts of DON metabolites haven't been considered in the regulatory limits fixed by food agencies due to the lack of data on their absorption and toxicity (Maresca 2013).

DON was stable during food processing and storage at high temperatures (Sugita-Konishi *et al.* 2006). The extensive distribution of DON in agricultural commodities and its biological adverse effects in animals and human make it necessary to pay attention to the risk of consuming DON-contaminated foods. In the animals, swine are more sensitive to DON than mice, poultry, and ruminants due to species difference in the metabolism of DON (Pestka and Smolinski 2005). Even though studies have been made on the distribution of DON in animals, however, these studies performed are confined in mouse or a few organs of pigs (Döll *et al.* 2003; Maresca 2013). Further investigation of DON distribution in vast ranges of tissues in domestic livestock is necessary. Also, it is widely acknowledged that study on DON provides basis for further research on metabolism of the fusarium toxin and a physiological specimen-based diagnosis of intoxication (Dänicke and Brezina 2013).

Therefore, on the basis of recommended TDI of DON in human and the characteristic of enterophepatic circulation and glucuronidation detoxification of DON in pigs, doses of 250 and 750 μg kg^{-1} body weight were intravenously administrated into piglets respectively, and the tissue distribution of DON in pigs was investigated by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) and immunohistochemistry in our study.

2. Results

2.1. Clinical symptoms

5 to 10 min post-administration, the animals in group B exhibited distinctively abnormal behaviors, including rampaging, teeth grinding, chewing, excessive salivation, and vomiting. The duration of these behaviors varied considerably among individuals within the group. In group A, no abnormal behavioral symptoms were observed at 30 min after intravenous administration.

2.2. DON concentrations in the plasma and tissues

The calculated limits of detection (LODs) for DON in plasma and bile were 1 ng mL^{-1}; the LODs of DON in tissue samples including stomach, duodenum, jejunum, ileum, cecum, colon, rectum, mesenteric lymph nodes, liver, kidney, spleen, and muscles were all 2 ng g^{-1}. Limits of quantification (LOQs) in plasma and bile were 2 ng mL^{-1}, and 5 ng g^{-1} for the tissues mentioned above, respectively. Recoveries of DON ranged from 83.65–91.52% for plasma and bile, and 83.02–91.00% for tissues, respectively, depending on the fortification level. The coefficients of variation of intra-assay and inter-assay were acceptable and 2.35–6.88% and 3.22–3.77% for plasma and bile, 2.78–9.01% and 3.65–7.93% for tissues, respectively. The developed analytical method proved to be available for the detection of DON in plasma, bile and tissue samples of pigs.

DON concentrations in plasma, bile and tissue samples