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Toxicokinetics and tissue distribution of nivalenol in broiler chickens

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ABSTRACT

Nivalenol (NIV), a type B trichothecene mycotoxin, is mainly produced by the fungi of *Fusarium* genus, which naturally occurs in agricultural commodities. Consumers are particularly concerned over the toxicity and safety of NIV in food animal products. To evaluate the toxicokinetics and persistence of residues of NIV, NIV was administered intravenously (iv) or orally (po) to broiler chickens at a dosage of 0.8 mg/kg body weight. The concentration of NIV in the plasma and various tissues was detected using liquid chromatography tandem-mass spectrometry. The plasma concentration of NIV in broilers could be measured up to 24 h and 12 h after iv and po administration, respectively. The value of elimination half-life of NIV was 5.27 ± 0.82 h and 2.51 ± 0.88 h after iv and po administration, respectively. The absolute oral bioavailability was 3.98 ± 0.08%. NIV was detected in the intestine, kidney, muscle, heart and liver after po administration. Regarding tissue residues, largest quantities of NIV were found in the small intestine. These results suggest that NIV is absorbed from the gastrointestinal tract with low bioavailability and it has the ability to diffuse into various tissues of broilers.

1. Introduction

Mycotoxins are dangerous, naturally produced compounds that are ubiquitous in agricultural commodities. The Food and Agricultural Organization (FAO) of the United Nations has estimated that up to 25% of the world's food crops are significantly contaminated with mycotoxins (Rice and Ross, 1994). Due to their toxic properties and their resistance to heat treatment, the presence of mycotoxins in the food chain is potentially hazardous for animal and human health. Humans and animals can be directly exposed to mycotoxins by consuming contaminated food and livestock feed, respectively. Different mycotoxins have been found to contaminate food products such as meat, milk and eggs (Sypecka et al., 2004; Hussain et al., 2010; Afshar et al., 2013; Herzallah, 2013).

Trichothecenes is a mycotoxin family known to have a strong impact on the health of both humans and animals (Sudakin, 2003). Trichothecenes are a group of mycotoxins commonly found in cereals such as wheat, barley, oats and maize worldwide. These mycotoxins are mainly produced by *Fusarium* species of fungi. The relevant members of this family include T-2 toxin, diacetoxyscirpenol (DAS), deoxynivalenol (DON), fusarenon X (FX) and nivalenol (NIV) (D'Mello et al., 1999; Eriksen and Pettersson, 2004). A number of previous reports have shown that they are both highly toxic and detected with relative frequency in the food chain (Tanaka et al., 1988; Binder et al., 2007; Haschek and Beasley, 2009; Broekaert et al., 2015).

NIV (3,4,7,15-tetrahydroxy-12, 13-epoxytrichothec-9-en-8-one) is mainly produced by *Fusarium graminearum*, *F. crookwellense* and *F. nivale* and frequently occurs with FX and DON in crops such as wheat, barley and maize (IARC, 1993; Eriksen and Alexander, 1998; Placinta et al., 1999). Recent reports have shown that NIV occurs in cereal-based products in European countries (Gottschalk et al., 2009; Ibanez-Vea et al., 2012; Montes et al., 2012), Brazil (Del Ponte et al., 2012), Japan (Tanaka et al., 2010), Southeast Asia (Yamashita et al., 1995) and China (Luo et al., 1990). The amount of NIV in cereal products may vary considerably among different countries across the world (from 20 to 60 μg/kg in France, to 584–1780 μg/kg in China) (Hsia et al., 2004).

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NIV is reported to bind to the ribosomal peptidyl transferase site and to inhibit protein and DNA synthesis. Consequently, exposure results in decreased cell proliferation (Shifrin and Anderson, 1999). In addition, several early reports have focused on possible apoptotic mechanisms induced by exposure to other mycotoxins (Oh et al., 2001; Miura et al., 2002; Sutjarit et al., 2014). NIV can cause toxicity in animals with clinical signs related to gastrointestinal erosions, nephropathy, reduction of feed intake and cytotoxicity (D' Mello et al., 1999; Concovac et al., 2003; Fornelli et al., 2004). In addition, NIV has been reported to induce adverse effects, particularly apoptosis, in organs containing actively dividing cells such as the small intestine, thymus, spleen, bone marrow and testes. It also reduces proliferation in mitogen-stimulated human lymphocytes, as observed for other trichothecenes (Ohta et al., 1978; Forsell and Pestka, 1985; Miura et al., 1998; Poapolathep et al., 2002, 2004). The adverse effects of NIV in broiler chickens have been previously reported as reduced feed consumption, gizzard erosions and reduced relative liver weight (Hedman et al., 1995).

Although the toxicokinetics of several trichothecenes have previously been studied (T-2 toxin, HT-2, DON and FX) (Gauvreau, 1991; Dänicke et al., 2004; Goyarts and Dänicke, 2006; Poapolathep et al., 2008; Osselaere et al., 2013; Saengtienchai et al., 2014; Sun et al., 2015), to date, the toxicokinetics of NIV is incompletely understood. Regarding our previous investigations, we reported the value of toxicokinetic parameters including the elimination half-life, it was 1.36 h for zearalenone, 1.25 h for DON and 1.13 h for FX in broiler chickens (Poapolathep et al., 2008; Buranatragool et al., 2015, Pralatnet et al., 2015). Limited data are available and toxicokinetic profile of NIV has been reported in pigs and mice only (Hedman et al., 1997; Poapolathep et al., 2003). As NIV contamination mainly occurs in crops, poultry might be one of the main food-producing animal targets for NIV toxicity.

According to our knowledge, there are no published investigations on toxicokinetics and tissue residues of NIV in broiler chickens (Kongkapan et al., 2015, in press). The objectives of this study were to investigate the toxicokinetic characteristics and effect on tissues of NIV in broiler chickens following a single intravenous (iv) and oral (po) administration at a dose of 0.8 mg/kg body weight.

2. Materials and methods

2.1. Toxins and chemicals

Nivalenol (NIV) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chemicals of analytical grade (methanol and acetronitrile, ACN) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Purified water was produced using the Milli-Q water purification system from Millipore, Inc (Bedford, MA, USA). The solutions for iv and po administration were prepared at a concentration of 2 mg/ml by dissolving NIV standard with 0.01 M phosphate buffer saline pH 7.4.

2.2. Animals

Forty, 3-week-old-female broilers, weighting 1.2–1.5 kg, were obtained from commercial chicken farm (Charoen Pokphand Foods Public Company Limited.), located at Nakhon Ratchasima Province, Thailand. The experimental animals were housed in individual stainless-steel cages at the Laboratory Animal Facility, Faculty of Veterinary Medicine, Kasetsart University. They were acclimatized to the environment for 1 week. The animals were fed with a commercial diet (mycotoxin free) and water *ad libitum*. The feed was considered mycotoxin free after analysis for the presence of mycotoxins by a validated multi-mycotoxin liquid chromatography tandem mass spectrometry (LC-MS/MS) method (Ren et al., 2007). This study was ethically approved by the Animal Ethics Research Committee of the Faculty of Veterinary Medicine, Kasetsart University.

2.3. Experimental design for toxicokinetic study

Ten broiler chickens were randomly assigned to two treatment groups (n = 5), using slips of paper marked with the numbers 1 to 10, selected blinded from a box. After overnight fasting, group A was administered with NIV by a single iv bolus injection (0.8 mg/kg bw) into the right wing vein. Group B was given NIV by po (0.8 mg/kg bw), this was administered directly into the chickens' crop using a plastic tube attached to a syringe.

Blood samples were collected from the left wing or tibial veins of each chicken with heparinized syringes at 0, 5, 15 and 30 min and 1, 2, 4, 6, 8, 12 and 24 h after NIV administration. The plasma was separated by centrifugation at 1968 g for 15 min and immediately frozen at −20 °C until analysis.

2.4. Experimental design for tissue residue study

Twenty-five broiler chicken were administered NIV orally at a dose of 0.8 mg/kg bw (according to the procedure above described). Five broiler chicken served as controls and were orally administered with 0.01 M phosphate buffer saline pH 7.4. Animals were sacrificed with a lethal iv injection of thiopentone sodium at a dose of 20 mg/kg bw. Tissue samples, including liver, kidney, heart, muscle, small intestine and excreta, were collected at 1, 3, 6, 12 and 24 h after po administration (n = 5 for each point). All samples were frozen at −20 °C until analysis.

2.5. Extraction and clean up

One milliliter of plasma or 5 g of excreta or tissue were used for the analysis. One milliliter of phosphate buffer (0.1 M, pH 7.4) was used for tissue homogenization (5 g of each tissue) with an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington, DE, USA). Extraction of NIV from plasma, tissue and excreta was performed as previously described (Poapolathep et al., 2003). Briefly, 3 mL of acetonitrile–water (3:1, v; v) and 2 g of ammonium sulfate were added to the mixture before vortexing for 30 s and shaking for 15 min. The ACN fraction was separated by centrifugation at 1968 g for 15 min. These extraction steps were then repeated for 2 additional cycles. The supernatant fractions were combined and purified using the solid phase extraction cartridge (C18 Sep-pak silica cartridge) (Waters Corp., Milford, MS). The eluate was completely evaporated under a nitrogen stream at 40 °C on a heating block. The residue was re-dissolved with 200 μl of methanol–water (1:4, v/v) with 5 mM ammonium acetate (Broekaert et al., 2014) and then injected into a 0.22 μm syringed filter before being analyzed by liquid chromatography tandem-mass spectrometry (LC-MS/MS).

2.6. LC parameters

LC analysis was performed using an Agilent 1200 series system consisting of a binary-high pressure gradient pump, a vacuum solvent degassing unit, an automatic sample injector and a column thermostat (Agilent Technologies, Waldbronn, Germany). Separation was achieved by a ZORBAX Eclipse Plus RRHT C18 column (50 × 2.1 mm, 1.8 μm particle size) (Agilent Technologies, Palo, Alto, CA, USA). The column was maintained at a temperature of 40 °C. The LC mobile phase program consisted of a binary gradient of the 5 mM ammonium acetate in 0.2% acetic acid (mobile phase A) and methanol (mobile phase B). The composition started out at 95%