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Fate and tissue depletion of nivalenol in ducks

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Abstract

Nivalenol (NIV), a mycotoxin belonging to the trichothecenes type B group, has recently been identified as causing one of the more potent toxicities among mycotoxins of this group. The purpose of this study was to clarify the toxicokinetics, and residues of NIV in ducks. Then, NIV was administered intravenously (iv) or orally (po) to ducks at a dosage of 0.8 mg/kg body weight. The concentrations of NIV in plasma and various tissues were quantified using liquid chromatography tandem-mass spectrometry. The plasma concentrations of NIV were measurable up to 12 h after iv and po administrations, respectively. A non-compartmental model was used to describe the toxicokinetics of NIV in ducks. The values of elimination half-life and volume of distribution were 2.24 ± 0.34 h and 1081.87 ± 306.56 ml/kg, respectively, after iv administration. The absolute oral bioavailability was 8.91 ± 1.69%. NIV was measurable in the vital organs after po administration. These results suggest that NIV is not favorably absorbed from the gastrointestinal tract, but it has the ability to penetrate into the various tissues of ducks.

Key Words: nivalenol; toxicokinetic; residue; duck

Introduction

Nivalenol (NIV; 12, 13-epoxy-3,4,7,15-tetrahydroxytrichothec-9-en-8-one), a type B trichothecene mycotoxin, is a by-product of fungal metabolism, which has a ketone group at C-8 position of 12, 13-epoxy-trichothecene. NIV is mainly produced by various species of *Fusarium* including, *Fusarium graminearum*, *F. crookwellense* and *F. nivale*[10,27]. NIV was first isolated from the metabolites of *F. nivale* by Tatsuno *et al.* (1968)[24], and NIV-producing strains of *F. graminearum* are primarily found in Japan, Australia and New Zealand[1,9,13]. These fungi contaminate in agricultural commodities[10,11]. The European Food Safety Authority (EFSA) has issued guidelines on the risk to human and animal health related to the presence of NIV in food and feed in the

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form of a tolerable daily intake (TDI) of 1.2 μg/kg bw/day[3] whereas, the Food Safety Commission in Japan (FSCJ) has established a TDI of 0.4 μg/kg bw/day[7]. Trichothecene mycotoxins, non-protein toxins, including NIV are highly cytotoxic to eukaryotic cells and act by inhibiting protein and DNA syntheses[25,26]. Thus, they cause specific damage to the tissues containing rapidly proliferating cells such as lymphoid, hematopoietic tissues, skin, and gastrointestinal mucosa[15]. NIV can stimulate lipid peroxidation, alter cell membrane function and modulate immune responses. It activates mitogen activated protein kinases through the ribosomal stress response. In addition, as apoptosis has recently been recognized as one of the major mechanisms for trichothecene-induced toxicity, the induction of apoptosis by NIV has been studied in animals[16,17,19].

To date, although the toxicokinetics of several trichothecenes have previously been studied (T-2 toxin, HT-2, DON and FX)[2,5,6,20–22], the toxicokinetics of NIV is only partially understood and limited data are available. Over the past, we demonstrated the toxicokinetic characteristics of NIV in mice and chickens[12,18,19]. Its toxicokinetic profile was reported in pigs[8]. The objectives of this study were to investigate the toxicokinetic characteristics and tissue depletion of NIV in ducks following single intravenous and oral administrations at a dose of 0.8 mg/kg body bw.

Materials and Methods

Chemicals and reagents: Nivalenol (NIV) and other chemicals 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.

Animals: Forty, 4-week-old male ducks (Cherry Valley; average weight 1.7 ± 0.18 kg) were obtained from a commercial duck farm (CP group Co., Saraburi Thailand). The experimental birds were housed in individual stainless-steel cages at the Laboratory Animal Facility, Faculty of Veterinary Medicine, Kasetsart University. Birds were acclimatized to the environment for 1 week prior to the commencement of the study. The birds were fed with a commercial diet and water *ad libitum*. This study was ethically approved by the Animal Ethics Research Committee of the Faculty of Veterinary Medicine, Kasetsart University.

Toxicokinetic: To obtain the toxicokinetic data, ten ducks were randomly divided into two groups ($n = 5$). After overnight fasting, each group was administered NIV iv (through wing vein) or po at a dosage of 0.8 mg/kg bw. The dosage of NIV in this experiment was determined based on our previous publication in broiler chickens[12]. Blood samples (2.0–2.5 ml) were collected from the left wing or caudal tibial veins of each duck with heparinized syringes at 0, 5, 15 and 30 min and 1, 2, 4, 6, 8, 12 and 24 h after NIV administration. Animals were sacrificed with a lethal iv injection of thiopentone sodium at a dose of 20 mg/kg bw. The plasma was separated by centrifugation at 1,968 g for 15 min and immediately frozen at −20°C until analysis.

Tissue residues: Thirty ducks were administered NIV orally at a dose of 0.8 mg/kg bw (according to the procedure described above). Five ducks served as controls and were po 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 colon content, were collected at 1, 3, 6, 12 and 24 h after p.o. administration ($n = 5$ for each point) whereas the excreta was collected at 0–1 h, 1–4 h, 4–8 h, and 8–12 h. All samples were frozen at −20°C until analysis.

Extraction and clean up: Extraction of NIV from plasma, tissue and excreta was performed as previously described[18]. Briefly, 1 ml of plasma or 5 g of each homogenized tissue or excreta was extracted with 3 mL of ACN–water (3 : 1, v ; v). 2 g of ammonium sulfate were added to the mixture before vortexing for 30 seconds and shaking for 15 min. The ACN fraction was separated by centrifugation at 1,968 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 elute 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, and then injected onto a 0.22 µm syringed filter before being analyzed by liquid chromatography tandem-mass spectrometry (LC-MS/MS) (Agilent Technologies, Waldbronn, Germany).

LC parameters: LC analysis was performed as previously described[12]. Separation was achieved by a ZORBAX Eclipse Plus RRHD 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 limit of quantification (LOQ) of NIV in the plasma was 1.0 ng/ml. The corresponding respective LOQ values for NIV in the liver, kidney, heart, muscle, small intestine, colon content and excreta were 2.5–5.0 ng/g.

MS parameters: Mass spectrometry was performed using an Agilent Technologies 6460 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source and Agilent Mass Hunter Workstation Software version 1.2. ESI-MS/MS was operated at unit mass resolution in multiple reactions monitoring (MRM) negative ion mode with the following settings: nebulizer gas pressure (NEB): 50 psi, gas flow 8.0 l/ml, gas temperature 320°C and capillary voltage: −3500 V. The molecular ions and fragments were as follows: Q1: m/z 371.1, Q3(1): m/z 281.1, CE(1): 10 eV, Q3(2): m/z 59.1 and CE(2): 8 eV.

Method validation: Validation was performed to assess the efficiency of the LC-MS/MS method by investigating its selectivity, sensitivity, accuracy, and precision in accordance with the European Commission regulation for the performance of analytical methods[4]. The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. Linear regression analysis was conducted for NIV under the optimized LC-MS/MS conditions. Recovery, precision and accuracy were determined within day by analysing seven replicates containing NIV at three different quality control levels (10, 50 and 200 ng/ml or ng/g). The inter-day precisions were determined by analysing quality control samples on 5 days (one batch per day). To evaluate recovery, NIV was added to samples of blank plasma or homogenized tissue to yield final NIV concentrations of 1, 5, 10, 50 100 and 500 ng/ml or ng/g, respectively. The spiked samples were then analysed in duplicate as described in the extraction/clean-up procedures. The average (±SD) recoveries for NIV in the plasma, liver, kidney, heart, muscle, small intestine, colon content and excreta were, 97.1 ± 2.2%, 96.6 ± 4.8%, 96.4 ± 4.7%, 93.8 ± 5.3%, 94.5 ± 6.2%, 95.5 ± 4.7%, 86.63 ± 9.3% and 88.9 ± 4.2%, respectively. The intra- and inter-day precisions of spiked samples ranged from 2.6 to 6.9% and 3.8 to 7.9%. The calibration standard concentrations were prepared in duplicates by spiking the working standard solution into blank samples to yield final concentrations of 1, 5, 10, 50, 125, 500 and 1000 ng/mL or ng/g, respectively. The r^2 values of NIV calibration curves were higher than 0.9996.

Toxicokinetic parameters calculations: The concentration of NIV in experimental ducks vs time was described by a non-compartmental model using WinNonlin software (version 5.3.1). $C_p^{\ 0}$ was the peak concentration at the initial time, C_{max} was peak plasma concentration, T_{max} was time at peak plasma concentration, AUC was the area under the curve, V_d was the volume of distribution, $t_{1/2\lambda}$ was the elimination half-life, Cl was the plasma clearance, MRT was the mean residence time, Vd_{ss} was the volume of distribution at steady state. The oral bioavailability (F) was calculated using the following equation:

$(\%)F_{(po)} = (\text{AUC po}) / (\text{AUC iv}) \times 100$

Statistical analysis: Toxicokinetic variables were evaluated using the student's t-test to determine statistically significant differences between the treatment groups (iv vs po). Both toxicokinetic parameters and NIV plasma concentrations are presented as means ± SD (normality tested by Shapiro-Wilk test). All analyses were conducted using GraphPad InStat (GraphPad Software, La Jolla, CA, USA). Differences were considered significant if $p < 0.05$. The tissue residues of NIV are shown as the mean (± SD, n = 5) of the values for the ducks sampled. The ANOVA test has been used to evaluate the statistically significant differences in the NIV concentrations among the different tissues. A value of $p < 0.05$ has been selected as significant.

Results

Plasma Toxicokinetic

NIV was detectable in the plasma of ducks following single iv or po administration. The semilogarithmic plots of the mean (±SD) plasma concentration-time curve of NIV at a dosage of 0.8 mg/kg bw in ducks following iv and po administrations are shown in Fig. 1. NIV was measurable from 5 min to 12 h, after iv and po administrations. The mean plasma vs time curve profiles showed a lower concentration of NIV after po as compared to iv administration. This is also reflected by the AUC values for iv vs po, reporting a $F\%$ value of about 8.9%. The toxicokinetic parameters are shown in Table 1. Following iv administration, the value for the $t_{1/2\lambda}$ of NIV (2.24 ± 0.34 h) was shorter, but not statistically so, than that obtained after po administration (6.47 ± 4.24 h). The Cl value was low (331.98 ± 66.38 ml/h/kg) while the V_d was wide (1081.87 ± 306.56 ml/kg). The significant differences ($p < 0.05$) between the groups were found in *Vd, Cl*, *AUC* and *MRL* parameters (Table 1).

Tissue residue

The LC-MS/MS profile for various tissues, including the kidney, muscle, heart and small intestines, showed that NIV was measurable from 1 h to 24 h in all examined tissues, whereas it was detected from 1 h to 12 h in the liver after po administration (Table 2). The maximum level of NIV was 429.48 ± 170.94 ng/g at 1 h in the small intestine of ducks.

In excreta, NIV was detectable up to 24 h after oral administration. The highest amount of NIV was found between 1 h to 4 h, and was detected until 24 h after po administration (Fig. 2). The mean (±SD) values of residues in examined tissues of ducks are reported in Table 2.

Discussion

In the present study, at a dosage of 0.8 mg/kg bw, no clinical adverse effects were noticed after oral administration. Regarding the toxicokinetic study, the results revealed that NIV is rapidly absorbed into systemic circulation after po administration. NIV appears to be rapidly detectable, and eliminated in the duck. The elimination half-life value ($t_{1/2\lambda}$) was longer in the po group than in the iv group, but the difference was not statistically significant. The $t_{1/2\lambda}$ of NIV obtained in ducks was shorter than those reported in broiler chickens (5.27 ± 0.82 h)[12] and mice (14.34 h)[18]. The present study demonstrated

Table 1. Mean ± SD value of the toxicokinetic parameters of nivalenol following iv and po administration at a dosage of 0.8 mg/kg bw in ducks (n = 5 each)

Toxicokinetic parameters (units)	Value		Value in broilers (*Kongkapan et al., 2016*)	
	iv	po	iv	po
$t_{1/2\lambda}$(h)	2.24 ± 0.34	6.47 ± 4.24	5.27 ± 0.82	2.51 ± 0.88
Kel (h^{-1})	0.32 ± 0.04	0.16 ± 0.10	–	–
T_{max}(h)	–	1.80 ± 1.30	–	2.4 ± 0.89
C_{max} (ng/ml)	–	72.39 ± 22.12	–	62.56 ± 30.86
$C_p^{\ 0}$ (ng/ml)	3974.32 ± 1446.64	–	10,409.66 ± 4249.17	–
AUC_{last} (h ng/ml)	2444.61 ± 486.54	274.25 ± 67.03*	7026.87 ± 1246.97	279.98 ± 108.53
AUC_{inf} (h ng/ml)	2486.95 ± 484.94	340.41 ± 66.53*	–	–
$Vd^{\S}$ (ml/kg)	1081.87 ± 306.56	21596.47 ± 13153.34*	853.93 ± 136.29	9974.72 ± 3936.82
$CL^{\S}$ (ml/h/kg)	331.98 ± 66.38	2438.85 ± 572.36*	113.57 ± 18.95	2817.32 ± 1026.80
MRT (h)	2.10 ± 0.20	7.35 ± 3.16*	2.34 ± 0.39	3.29 ± 0.70
Vd_{ss} (ml/kg)	701.52 ± 175.52	–	336.42 ± 81.18	–
F (%)	–	8.91 ± 1.69	–	3.98 ± 0.08

Note: $t_{1/2\lambda}$ = elimination half-life; *Kel* = elimination rate constant;
T_{max} = time at maximum concentration; C_{max} = the maximum concentration;
$C_p^{\ 0}$ = plasma concentration at initial time; AUC_{last} = area under the curve from zero to 12 h; AUC_{inf} = area under the curve from zero to the last
V_d = volume of distribution, *CL* = clearance; *MRT* = mean residence time;
V_{dss} = volume of distribution at steady state;
F = oral bioavailability. §This data is divided for bioavailability when calculated for the po administration; $^{*}P < 0.05$ (statistically significant value if compared to the corresponding iv group)

Table 2. Mean ± SD residue concentrations of nivalenol (NIV) in various organs following po administration at a dosage of 0.8 mg/kg bw in ducks (n = 5 each)

Time (h)	NIV concentrations (ng/g)					
	Liver[a]	Kidney[b]	Muscle[c]	Heart[d]	Small intestine[e]	Colon content[f]
1	28.09 ± 12.67	18.74 ± 12.07	26.52 ± 6.34	21.28 ± 8.60	429.48 ± 170.94[a, b, c, d]	284.07 ± 200.56[a, b, c, d]
3	16.55 ± 5.81	31.06 ± 8.85	51.52 ± 24.72	80.16 ± 58.99	139.47 ± 121.08	1140.13 ± 651.83[a, b, c, d, e]
6	13.99 ± 8.09	32.08 ± 14.12	34.95 ± 16.13	14.89 ± 6.37	81.10 ± 52.03	258.51 ± 129.92[a, b, c, d]
12	9.30 ± 4.67	15.14 ± 11.77	12.69 ± 6.27	44.32 ± 25.14[a, b, c, e]	15.01 ± 3.89	23.01 ± 9.62
24	ND	6.69 ± 3.59	5.99 ± 2.53	83.12 ± 16.81[b, c, e, f]	10.18 ± 1.38	9.92 ± 1.55

ND = not detected, Superscript letters indicate the statistically significance difference ($p < 0.05$) among organs, a = liver; b = kidney; c = muscle; d = heart; e = small intestine; f = colon content.

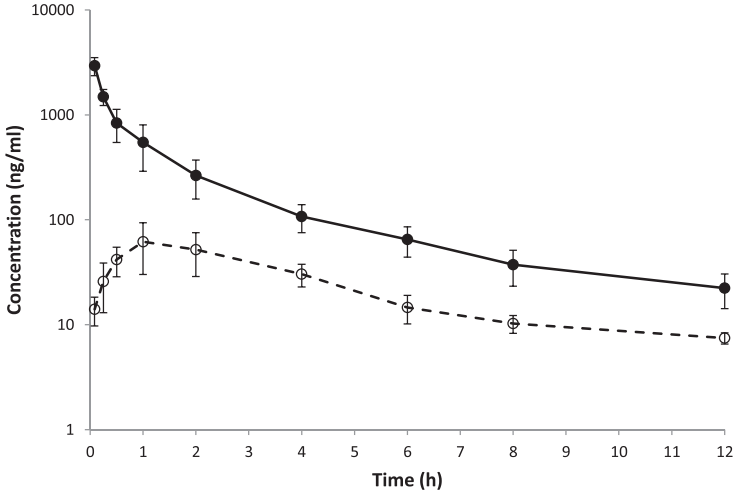

Fig. 1. Mean values (±SD) of nivalenol concentration in plasma of ducks at a dosage of 0.8 mg/kg b.w. (●) intravenous administration, and (○) oral administration (n = 5).

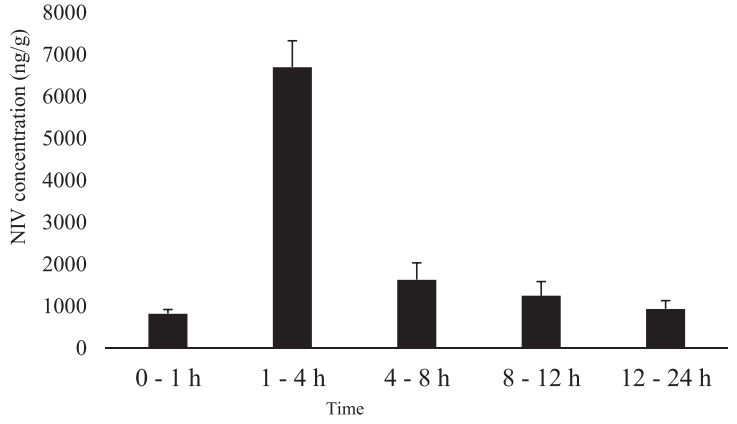

Fig. 2. Average of excreta concentrations of NIV after oral administration of 0.8 mg/kg bw in ducks.

a *Cl* value of 331.98 ± 66.38 ml.h/kg after iv administration. This value was greater than that reported in broiler chickens (113.57 ± 18.95 ml. h/kg)[12]. This might indicate that NIV is more efficiently eliminated by the kidney and liver in ducks compared to broiler chickens. Liver and kidney are important organs not only for the elimination of xenobiotic, but also for their metabolism. It is unclear however if the administered dose has impaired the normal functioning of liver and kidney. Following po administration, the maximum plasma concentration (Cmax) of NIV (72.39 ± 22.12 ng/ml) was reached at the time of maximum concentration (Tmax), 1.80 ± 1.30 h. The Cmax of NIV in ducks was similar to that reported earlier in broiler chickens (62.56 ± 30.86 ng/ml)[12]. The very low plasma concentration of NIV after po administration is indicative of the low ability for absorption in ducks, which relates well with its low oral bioavailability (8.9%). The oral bioavailability of NIV in ducks was twice that reported in broiler chickens (4%). These findings might indicate that NIV is more efficiently absorbed in the gastrointestinal tract of ducks. Trichothecenes undergo a variety of different metabolic reactions including hydrolysis to split off side groups, hydroxylation and de-epoxidation. NIV has been reported to be metabolized to a de-poxidated form by microorganism in the gastrointestinal tract in rats[14] but it was not detected in mice and pigs[8,18]. In this study, the detection of de-epoxynivalenol,

a metabolite of NIV, was not performed.

The residue findings showed that NIV was detectable in various tissues including the kidney, heart, muscle, small intestine and colon content from 1 h to 24 h while it was detected in the liver from 1 h to 12 h after po administration. The level of NIV was highest in the small intestines, followed by heart, muscle, kidney and liver. These results indicated that NIV had the ability to penetrate various tissues of ducks. The large amount of NIV found in excreta is in line with the large amount of NIV found in the colon content. Although a direct comparison between these values is not possible, it is likely that the amount of NIV found in excreta is mainly due to the un-absorbed fraction of NIV rather than the fraction eliminated via the kidney. In line with the present results, NIV has been shown to be mainly excreted in feces in mice, pigs and broiler chickens[8,12,18]. The maximum concentration of NIV that should be tolerated in poultry feed has not been established thus far. The European Food Safety Authority Panel on Contaminants in the Food Chain has established a TDI for NIV. In the present study, NIV was detected in a number of edible tissues. Based on the lowest TDI of NIV, concentrations in tissues used for meat (after an oral ingestion 0.8 mg/kg bw) do not exceed the TDI and should not be of concern for consumers' health. Indeed the edible tissue with the highest NIV content was the heart (0.08 μg/kg) and the consumer would have to eat over 120 kg of duck's heart to exceed the TDI value. These findings are in line with a previous study on tissue depletion in broiler chickens[12].

In conclusion, it showed that NIV was detectable in the plasma following both iv and po administrations. It was detected in largest amount in excreta and colon content, while in small amount in the other tissues of ducks after po administration. Based on toxicokinetic information, NIV is poorly absorbed orally from the gastrointestinal tract and rapidly eliminated in ducks via excreta. However, although in small amount NIV can penetrate into various tissues such as the small intestine, kidney, heart, liver and muscle. The results obtained in this study contribute to the knowledge of toxicokinetic characteristic and residues of NIV in the duck. Certainly, this study could pave the road to establish both the regulatory limits of NIV for human consumption and its ecological impact.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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